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INDUCING DROUGHT RESISTANCE IN CROPS

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INTRODUCTION

Drought probably is the major limiting factor in crop production in the sub humid and semi arid regions of the world. Within these regions are found the principal wheat producing areas of Canada, the United States, Argentina, Australia and Russia. Periodically, these areas are subjected to conditions of drought which may be so severe as to cause a virtual crop failure.

The importance of the problem of drought resistance in field crops has been increasingly recognized during recent years. Lack of drought hardiness in wheat, undoubtedly, is one of the major factors limiting production. The use of yield trials as an index for drought resistance has not proved entirely satisfactory under dry conditions because early varieties may have high grain yields, but they are drought escaping rather than drought resistant. Earliness may not be a desirable characteristic in wheat, since early varieties have a short period to grow and are likely to be injured by late spring frosts.

In the earlier part of this century, most research workers believed that drought resistant plants transpired at a low rate and their water requirements were small; while, in recent times, investigators believe that the ability of crop plants to obtain and conserve moisture under dry conditions is the criterion of drought hardiness.

The phenomenon of drought resistance is still not well understood and studies have been made in various directions to discover the physiological nature of the same. The present paper reports the results of investigations carried out in an attempt to induce drought resistance in crops.

REVIEW OF LITERATURE

The difficulty of obtaining a true concept of what is meant by drought resistance is complicated at the outset by the existence of two types of drought, each of which presents to the investigator a distinct series of problems. The type of drought that is best known and which has received the greatest attention in the past is that occuring at a time when the soil ceases to provide the plant with sufficient moisture to replace the loss through transpiration. Drought of this nature is commonly known as soil or edaphic drought. The second type, which has gained recognition only recently, is known as atmospheric drought. This is usually caused by hot dry winds, which may produce desiccation of the plant, even under conditions of relatively high soil moisture. Briggs and Shantz (1913) in their work on water requirements of plants, however, paid special attention to the soil type of drought.

DROUGHT ENDURANCE AT DIFFERENT STAGES OF PLANT GROWTH

Plants exhibit different capacities for enduring drought at various stages of their growth and development. The mature seed can undergo almost complete desiccation without apparent injury. This resistance appears to be manifested during the first few days following germination. Robbins (1917) germinated seeds of wheat and buckwheat six times, each time allowing the root and stems to grow to the length of the grain before stopping the process by dryness. He found that five repeated germinations were necessary before the percentage germination of these seeds was materially reduced. With the development of leaves, however, the plants became susceptible to severe injury by desiccation.

Brounov (1899) found that cereals were most susceptible to drought during the period of rapid growth of the culm prior to heading. He applied the term "Critical Period" to the stage at which the plant was most susceptible. Azzi and Beawerie (1922), Moliboga (1927), and Pullman (1905) have also studied the critical period in cereals and confirmed the findings to Brounov.

Aamodt and Johnston (1936) reported results of drought injury in four varieties of wheat at various stages of growth. They concluded that shooting stage was more critical than stooling, soft dough, and hard dough.

HARDENING PROCESS AS A MEANS OF INDUCING DROUGHT RESISTANCE IN CROPS

Increased resistance of plants to adverse conditions through external influences has been termed "hardening." According to MacDougal (1914) plants may be hardened by exposure to cold, restricting the water supply, growing in poor soils, by root prunning, or by watering with a dilute salt solution. Tumanov (1927) found that repeated wilting of sunflowers resulted in a "hardening" process analogous to that observed at low temperatures. Kondo (1931) observed the same phenomenon in soybeans as well as in sunflowers. He also found that plants grown under conditions of insufficient soil moisture received much less injury from exposure to severe

conditions of soil drought than plants grown under conditions of optimum soil moisture. Aamodt and Johnston (1936) observed a definite favourable influence of hardening induced by scant moisture supply to the soil or by limited period of atmospheric drought. Greater tolerance to drought in the case of both resistant and susceptible plants was found to be developed by hardening.

Chandler (1941) concluded that slow wilting or partial witholding of water over a long period of time increased resistance to cold. Salmon and Fleming (1918) and also Harvey (1918) demonstrated that less succulent plants harden more when grown in a soil having low moisture content. Newton and Brown (1931) observed greater reduction in moisture content of hardy varieties due to hardening. These findings are similar to those made by Martin (1930). Laude (1933) reported that winter wheat, rye, barley and oats could be hardened outdoors by natural weather conditions. This hardening effect, however, was gradually lost when the plants were placed in the green house.

Different organs of the same plant have been found to vary in their degree of drought resistance. Tumanov (1927) observed that leaves of *Sorghum* withdrew water from the stem under stress of soil drought, while the leaves of buckwheat lacked this ability. He also found that the aerial parts of alfalfa plant exhibited less of drought resistance than its crown and roots. Maximov (1929) has cited instances where leaves of cereals drew water from the inflorescence when exposed to artificial winds. He also reported that, when the leaves were removed before exposure of the plants to the wind, the inflorescence had a much higher water content following exposure, than inflorescences of plants with the leaves intact.

MATERIAL AND METHODS

These experiments were conducted at Kansas State College (U.S.A.) in the green house. The investigations included studies on wheat and corn. Four varieties of wheat, viz., Wichita, Pawnee, New Pusa 4 and New Pusa 52, and four of corn hybrids, viz., K 1784, K 1639, K 2234 and K 1830 were selected for experimentation.

The investigations were preferably carried out in the green house in between the fall of 1952 and the spring of 1954. This permitted conducting several experiments with repeated trials than could be possible under field conditions within the period available. Observations recorded herein were considered comparable to field ones as a definite correlation between laboratory and field results has been pointed out by Salmon *et al* (1918). In studying resistance to drought injury in inbred lines of maize, Heyne and Laude (1940) reported that essentially the same order of resistance will be found in the laboratory as in field observations.

The plants were raised in six-inch porous clay pots filled with well mixed air dried silt loam soil. Six plants of each variety were grown in one pot. The pots were randomised and the replicates rotated once a week on the green house table. Suitable soil moisture and other conditions were maintained for obtaining adequate plant growth. Plants subjected to soil drought were deprived of water as they reached the stage of development selected for the test, until the plants showed evidence of permanent wilting. They were kept in this wilted condition for three days. Water was then applied to the soil to bring it up to the field capacity. Plants usually recovered in full or in part. Survival was recorded by critical,

unbiased visual estimates, assigning hundred per cent recovery for a fully green recovered plant.

In case of the study of the hardening process as a means of increasing resistance, the number of pots was equally divided into two groups, after germination. One of these groups was given the normal care and supplied adequate moisture at all times and, consequently, was not hardened. The other group of pots was subjected to the hardening process by allowing the soil to dry out until the plants showed signs of permanent wilting, i. e., the stage when cell injury of the leaves was just beginning. The moisture in the soil at this stage was considerably below 20 per cent of the water holding capacity. Water was then added to the soil and the plants were allowed to regain their normal condition. This process was repeated several times until the plants reached the desired stage of growth. The soil was then wetted to field capacity and both the hardened and non hardened groups of plants were subjected to similar but prolonged treatment of artificial drought.

EXPERIMENTAL RESULTS

Hardening process as a means of inducing drought resistance in crops—Many plants have the inherent ability to adjust themselves physiologically to adverse conditions when exposed gradually to unfavourable factors. This phenomenon is well illustrated on testing the varieties of winter wheat for resistance to low temperatures. The inherent potentialities of varieties to withstand injury from exposures to low temperatures are clearly differentiated after a hardening process. It has also been pointed out that a limited exposure of plants to drought developed in them a greater degree of tolerance to resist more severe exposures later on. Studies on the influence of hardening process on varietal reaction to drought were carried out, using four different varieties of wheat and corn at different growth stages.

Averages of observations made in these series of experiments to determine the differences in hardened and non hardened wheat plants have been presented in table I.

TABLE I

Relative recovery of wheat plants exposed to drought with and without previous hardening

Experiments		Percentage recovery	
		Non Hardened	Hardened
1	...	30·0	70·0
2	...	25·0	85·0
3	...	21·0	90·0
	Average	25·3	81·7

These data clearly indicate the greater resistance of hardened plants as shown by high percentage of recovery (approximately 82 per cent) compared to those that did not receive any hardening treatment. Average recovery in these latter cases was in the vicinity of 25 per cent only (Table I).

Other tests were conducted to study the relative effect of the hardening process at different stages of plants viz., four weeks, six weeks, and boot stage. The data presented in table II are the averages of two experiments conducted at different times in 1953. At the four weeks' stage, the recovery ratio of the non-hardened to the hardened plants was 1 : 3. In case of non hardened plants of Variety N.P.25 there was, however, no recovery. Wichita and Pawnee varieties responded well to both hardened and non-hardened conditions. There was no visible difference in between the behaviour of these two varieties.

TABLE II

Percentage recovery of four varieties of wheat exposed to drought at different stages of growth with and without previous hardening.

Wheat varieties	Age of plant					
	4 Weeks		6 Weeks		Boot stage	
	Hardened	Non-hardened	Hardened	Non-hardened	Hardened	Non-hardened
Wichita	74·1	32·2	59·4	18·4	90·2	31·6
Pawnee	62·2	35·0	50·6	5·6	33·3	21·6
N.P.4	49·4	5·2	34·7	3·8	0	0
N.P.52	38·1	0	33·1	0	0	0
Average	59·9	18·1	44·4	6·0	30·9	13·1

L.S.D. — 16·1

The rates of recovery in between non hardened and hardened plants went up to 1 : 6 when six week old plants were treated. Wichita and Pawnee were still in the upper brackets, while N.P. 4 was next, and N.P. 52 lowest in the order of recovery. The survival ability of plants at the boot stage was considerably reduced except in case of Wichita. The recovery in non hardened plants was only 13 per cent, while it was 31 per cent in case of hardened plants. N.P. 4 and N.P. 52 did not survive at all in both hardened and non hardened conditions.

Statistical analysis indicated that percentage recovery was significantly greater for the hardened plants relative to unhardened. Survival in Wichita was significantly greater, followed by Pawnee, which too was different from the two New Pusa varieties not differing significantly.

Results obtained following hardening of corn plants are shown in table III. The rates and trends of rccovery are very much like wheat.

TABLE III

Comparative recovery of corn plants when exposed to drought with and without previous hardening

Experiment		Non hardened	Hardened
1	...	26·0	62·0
2	...	19·0	70·0
	Average	22·5	66·0

Reaction of plants at the age of three weeks did not indicate as much difference between the hardened and non hardened plants, as at later stages. Possibly in this short period of time, plants are unable to build up physiological characteristics necessary for hardening. These plants were subjected to hardening only twice. At the age of four-weeks, however, 40 per cent of the hardened plants recovered, against only 12 per cent in the unhardened. The data are presented in table IV.

TABLE IV

Percentage recovery of four strains of corn when exposed to drought at different stages of growth with and without previous hardening.

Strains	Age of plant			
	3 weeks		4 weeks	
	Non hardened	Hardened	Non hardened	Hardened
1	2	3	4	5
K. 1784	9·1	22·2	8·5	35·0
K. 1639	30·3	49·7	20·0	47·8
K. 2234	45·6	51·8	21·0	69·4
K. 1830	26·9	19·7	0	10·0
Average	27·9	35·8	12·4	40·5

L.S.D. 16·8 for the differences between the means of the strains.

Strain K. 1830 was the poorest in recovery and lagged behind at all stages and in all treatments. Percentage recovery was greater in K 2234 than the other strains in both hardened and non hardened conditions, followed by K 1784 and K 1639 respectively. Hardened plants were significantly superior to non hardened plants irrespective of their age. Inspite of the low degrees of freedom in the analysis of variance K 2234 and K 1639 definitely proved superior to the other two strains in regard to percentage recovery at the ages of three and four weeks respectively.

DISCUSSION

The hardening process consisted in raising plants with scant moisture supply. Plants grown under such conditions were assumed to have been hardened. One of the important features of the hardening process observed was a higher percentage of recovery in the case of hardened plants (82 per cent) and lower recovery (25 per cent) in the case of non hardened plants. Percentage recovery was decidedly greater in hardened plants irrespective of their stage of growth. Response of hardened plants was essentially similar both in the case of wheat and corn. Varietal differences in susceptibility were all the same maintained even after the hardening process. Aamodt and Johnston (1936) observed that hardening of the plants before exposure to drought soil or atmospheric caused greater tolerance in both resistant and susceptible variteties of wheat. The results of the present investigation are in conformity with those obtained by Kondo (1931). He found that plants grown under conditions of insuficient soil moisture suffered considerably less injury from exposure to severe conditions of soil drought than plants grown under conditions of optimum soil moisture. Julander (1945) reported that plants grown under dry conditions survived a much longer exposure to heat before being killed than did plants grown with sufficient water e. g., four hours of high temperature produced complete killing in the case of watered bluestem, whereas 16 hours were required to kill plants that had been hardened by drought. Similar observations were made by Heyne and Laude (1933), who found that the heat resistance of seedlings, kept in the dark for 12 to 18 hours, increased significantly by an exposure to light for as short a period as one hour. Similar results were also obtained by Levitt (1951).

It may be assumed that plants grown under stress and scant moisture become less succulent and, consequently, the proportion of sugars and total solids in the tissues increases. This increase in the proportion of sugars makes the plant hardy. Newton and Brown (1931) observed a greater reduction in moisture content of hardy varieties due to the process of hardening. According to Laude (1933) the hardening effect could be lost under certain conditions. He found that winter wheat, rye, barley, and oats were hardened outdoors by natural weather conditions. This effect was gradually lost when the plants were brought to the green house. Similar results were obtained in a recent study of resistance of winter wheat varieties to heat and cold by Worf (1953).

SUMMARY

(1) A test of drought resistance in hardened and non hardened wheat plants indicated higher recovery in hardened plants.

(2) The study of the relative effect of hardening process to drought resistance in wheat plants at different stages of plant growth beginning at four weeks, six weeks, and at boot stage, indicated that hardened plants had better survival percentage at every stage of growth when subjected to drought.

(3) Similar tests to No. 1 and No. 2 in corn plants elicited a positive response. Hardened plants showed greater resistance to drought as compared to non hardened plants.

CONCLUSION

The hardening process consisted in allowing plants to grow with scant moisture supply. Plants grown under such stress conditions were assuemed to have been hardened. Hardened plants possessed greater resistance to drought in both resistant and susceptible types.

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FISH AND FISHERIES OF RANCHI LAKE, RANCHI (BIHAR)

By

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At present no authentic account of the Fish-fauna of Ranchi and its neighbourhood exists. As a matter of fact the whole Chotanagpur area leaves a virgin field for a scientific survey of its Fish-fauna and fisheries. Stray collections have been made in the past from few places of the Chotanagpur plateau but no systematic survey has yet been taken up of this vast hilly area which affords an interesting and scientifically important field for investigation and research (Hora 1938, Menon 1950).

Chotanagpur plateau makes the southern half of the State of Bihar and comprises the districts of Ranchi, Hazaribagh, Palamau, Dhanbad, Singhbhum, the Santhal Parganas, and the hills of Kharagpur. It is a continuation of the Deccan Plateau, situated between 83°–20″–87°–45″ longitude and 25°–15″–20°–20″ latitude and covers an area of 30,000 sq. miles.

The present paper deals with the fish and fisheries of a vast reservoir of water, the Ranchi Lake, which is the main source for the supply of fish-food for the Ranchi town. The Ranchi town is situated at an altitude of 2128 ft. above sea level. The Ranchi Lake is situated in the south-east corner of the town. It is not a natural one. It was formerly a mere depression of an insignificant size. Such a depression is a common feature in an area of granite and gnisse rocks. This owes its origin, partly to the removal of certain soluable minerals, such as felspar and partly to erosion by running water. The former initial depression was enlarged gradually by the immediate run off. The present outline of the lake is the outcome of the efforts of the late Lt. Col. Ousley, the then Commissioner of Chotanagpur. Ranchi lake covers an area of about 55 acres in the rainy season which gradually decreases to 54·10 acres during the summers. The average depth is 25 ft. in the wet months and declines to nearly 18 ft. during the dry months. The mud is locally known as 'Pankua', and is a fine clay with insignificant traces of silica. It is used by the local people as a fertilizer. The average annual rainfall is 57″.

SYSTEMATIC LIST

22 species of fishes belonging to 10 families have been recorded from the lake as listed below. The fishes were collected between the year 1956-58. The collections were made with the help of local fishermen. The fishes were caught by

means of cast and dragnets from different parts of the lake and at different depths, during the netting season, chiefly between October and February.

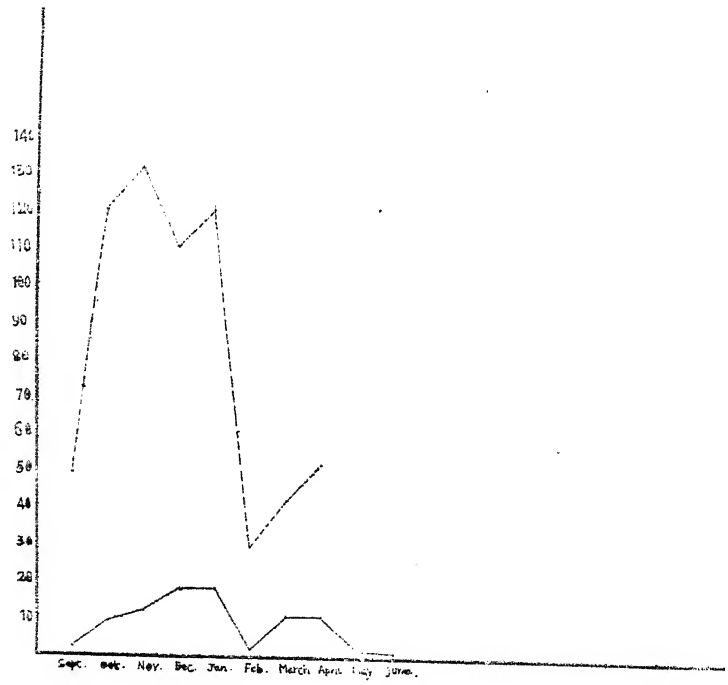
	Natural population	Local Names
Family :— Cyprinidae		
1. *Oxygaster bacaila* (Ham.)	F	Chalwa
2. *Puntus sarana* (Ham.)	C	Pothi
3. *Puntius stigma* (Cuv. and Val.)	C	
4. *Puntius ticto* (Ham.)	C	
5. *Cirrhina mrigala* (Ham.)	C	Mirgal
6. *Cirrhina reba* (Ham.)	C	
7. *Catla catla* (Ham.)	P	Catla
8. *Labeo rohita* (Ham.)	C	Rohu
9. *Labeo calbasu* (Ham.)	F	Calbasu
10. *Labeo bata* (Ham.)	F	Bata
11. *Barilius bola* (Ham.)	F	
Family :— Notopteridae		
12. *Notopterus chitala* (Ham.)	F	Palat
13. *Notopterus notopterus* (Pallas)	F	
Family :— Siluridae		
14. *Wallagonia attu* (Bloch)—Not found for the last 2 years.		
Family :—Bagaridae		
15. *Mystus aor* (Ham.)	R	Tengra
Family :—Heteropneustidae		
16. *Heteropneustes fossilis* (Bloch)	P	Getu
Family :—Claridae		
17. *Clarias batrachus* (Linn.)	P	Mangur
Family :—Ophicephalidae		
18. *Ophicephalus gachua* (Ham.)	C	Garai
19. *Ophicephalus punctatus* (Bloch)	C	
Family :—Gobidae		
20. *Glossogobius giuris* (Ham.)	C	Bula
Family :—Mastacembelidae		
21. *Mastacembalus armatus* (Lacep.)	F	Gunji
Family :—Amphipnoidae		
22. *Amphipnous cuchia* (Ham.)	F	Dugdigya.

P = Plenty ; C = Common F = Few ; R = Rare

No account of the existing fishery conditions of this region are available. Chauhan (1947) briefly described the fisheries of the Patna State only. The Ranchi lake is under the control of the Ranchi Municipality, which at present has given it on lease for ten years to an individual party at a rate of Rs. 2550 per year. The State Fisheries Department has no control over the lake.

The fishing activity lasts mainly during the dry months, between October and May. Fishermen belonging to Mallah, Oraon and Munda castes, who are especially trained, are employed. During the off season these fishermen engage themselves in agriculture. Spearing and poisoning is not allowed. During the rainy season only angling is allowed. The party charges Rs. 5 per rod per day. This fetches an income of Rs. 3000 to Rs. 4000 per month. During the wet months the netting is closed.

The netting starts from 2 a.m. and is completed latest by 7 a.m. 10 to 40 persons are employed at a time. These are employed on daily wages. Fishing boats are of medium size, plank-built and designed to carry 5-6 persons. These are 10′-12′ long, 2′-4′ wide and 15″-25″ deep. At a time 3-4 boats are used for netting. The main types of nets used are of two categories, *viz.*, cast nets (*majhalka jal*) and drag nets (*Mahajal*). The size of the drag net is 24 cubits wide (one cubit is $1\frac{1}{2}$″ ft.) and 100 cubits long with 1″ size mesh. Generally 10-12 persons carry this net. The number of hauling depends on the local demand. The per day yield from the lake during the netting season is about 5 mds. on an average, which is not sufficient to meet the demands of the people locally. The graph in Fig. 1 shows the monthly



1957—53

Fig. 1

------ Monthly yield of fishes (carps) from RANCHI LAKE.

——— Monthly yield of misllaneous fishes from RANCHI LAKE.

yield of carps and miscellaneous fishes during the year 1957-58. The maximum yield is in the month of November. The fishes caught every day are sold to local contractors, who transport them daily to the market for sale.

During the months between September and December the fish is sold at Rs. 2 to Rs. 3·50 per seer, but during the dry months when the yield gradually falls, the fish is sold at Rs. 3 to Rs. 4·50 per seer. No fish is exported outside the district as local demand itself is not fully met. As a matter of fact fishes are imported from Calcutta to meet the local demand. The fishes imported are Hilsa Bhekti, pomfret and a small percentaae of carps. The graph in Fig. 2, shows the monthly import

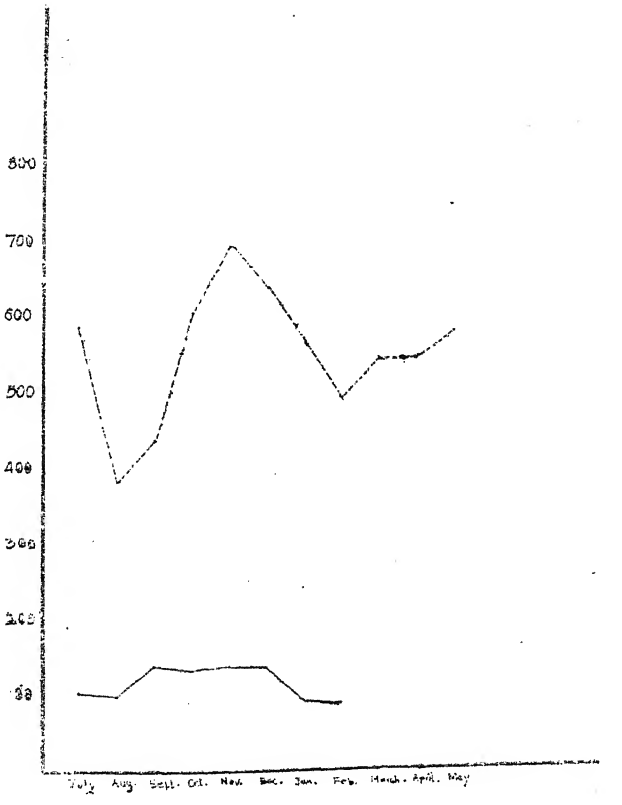


Fig. 2

------ Monthly import of fishes from CALCUTTA.

——— Monthly sale of fishes in RANCHI Town.

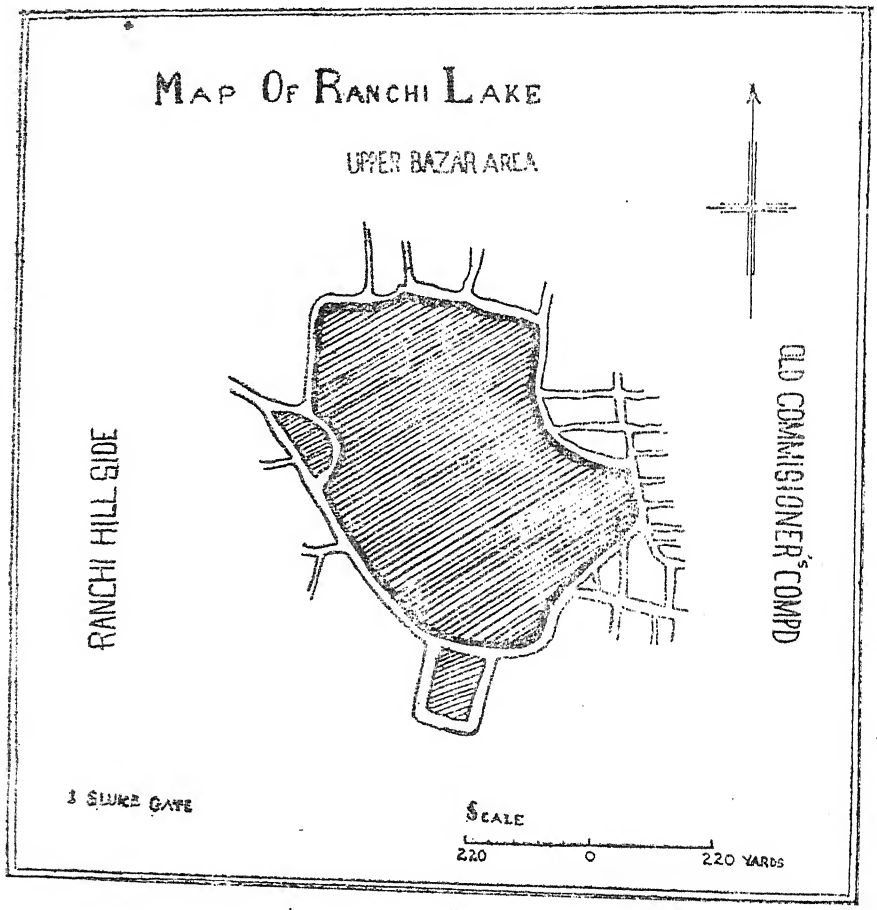
of fishes from Calcutta, and the monthly sale of fishes in Ranchi, during the year 1957-58.

The demand of fish scarcely exceeds the supplies in the town, and it is the experience of every fish-eater that it is difficult to get a regular supply of the good-fish in the market at a reasonable rate. The reason for this is the introduction of the middleman in transaction. The Fisheries Department can easily overcome this difficulty by decreasing the number of middleman and controlling the sale of the fish to the local merchants. The establishment of fishery centres will eliminate

the curse of middleman's profit, will standardise the price of different fishes and will also ensure a constant supply of good food-fish in the market.

FISH-CULTURE AND SEED RESOURCES

The supply of fries to the private parties is also an important factor in the development of fisheries. The Fisheries Department here gets the fries from Patna and they are not in a position to meet the local demand. A few tanks within the Municipal limits are stocked annually with fries. Certain local tanks (*Banas Pokhar, Duranda tank* and *Ghasi Pokhar*) which are under the management of the Fisheries Department, are stocked with fries of *Catla, rohu, cirrhina* and *calbasu*. Fingerlings of these carps are reared in the rearing ponds and also supplied to the D. V. C. The fries are measured by 'batty', the size of which is 2/7" dia. and 1" depth. One 'batty', usually contains 3000 fries. The size of the fries varies from $\frac{1}{2}$" to 1".



22 species of fishes have been recorded but only 11 of them are of economic value, judged from their size and weight attained or the bulk contributed by their abundance. *Labeo rohita, Labeo calbasu, Labeo bata, Catla catla, Cirrhina mrigala,* are the most common representatives of the major carps. *Catla* catla attains the maximum

weight of about $5\frac{1}{2}$" seers. Other carps average between 3/4—$3\frac{1}{2}$ seers. Important live fishes of food value are *Heteropneustis fossilis, Clarias batrachus, Ophicephalus punctatus* and *Ophicephalus gachua*. Among other varieties of fishes *Barbus* contrbiute the major bulk.

A full appraisal of the fishery potentialities connected with the Ranchi Lake have been dealt with in thispaper. Although Ranchi Lake covers a wide area yet its fish resources are very meagre as compared to the bulk of water it contains. It is advisable that Ranchi lake be taken over by the State Fisheries Department and only then its resources could be exploited to the maximum. Ranchi being the principal town of Chotanagpur it is rapidly growing in to an industrial city with its population increasing fast. It offers a good prospect for the development of its fishery resources and there are good opportunities. Considering the great need for increased food-fish production with the increasing demand of the people, the following surmises could be tentatively offered :—

(1) The state Fisheries Department should encourage the Co-operative and Private enterprises in rearing ponds.

(2) The Fisheries Department be equipped with mobile packing and transport units for marketting and transport of fries.

(3) Cold storage depots be set up for stocking purpose.

(4) Extension and propaganda work be undertaken for popularising fish-food.

(5) Preference be given to genuine fishermen Co-operative Societies for marketting fish.

(6) Arrangements for the rescue of the lake, from the polluted water discharged into the lake by the drains from the Upper Bazar area, be taken up, otherwise it would effect the mortality of the fishes.

(7) Steps be taken for the preservation, transport and marketting to be systematised to ensure a regular supply of wholesome fish to the new industrial population which is bound to grow up in the region.

SUMMARY

A preliminary study to assess the commercial yield of the fishery populations of the Ranchi Lake has been made. It has been found that the yield of the fish from the lake is nery meagre compared to its bulk of water. The reason being that it is controlled by a private party, which does not expoit its resources on a scientific basis. 22 species of fishes have been recorded—only 11 are of economic value. Out of the species mentioned here. *Notopterus chitala* is very rare, *Wallagonia attu* has not been found for the last two years, but it has been recorded previously and *Mystus aor* is also rarely found. The reason for the extinction of these fishes is not known. Data relating to the monthly yield of fish from the lake, monthly sale of fish in the market and monthly import of fish from Calcutta, during the year 1957-58, has been represented graphically.

ACKNOWLEDGMENTS

We are grateful to Principal, College of Science for his kind and constant encouragement; to Dr. K. C. Bose, Head of the Department, for his valuable suggestions and to the Assistant Fisheries Development Officer, Ranchi, for supplying certain valuable data.

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HISTOCHEMICAL STUDIES OF THE LIPOIDS IN THE DEVELOPING EGGS OF THE COMMON ENGLISH TROUTS 'SALMO TRUTTA AND SALMO IRIDEUS'.

III. PHOSPHOLIPINES : THEIR LOCATION AND ROLE

By

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INTRODUCTION

This is the third paper of the series and deals with phospholipines. They are almost universally present in animal tissues, and are believed to play an important role in lipoid metabolism. Although biochemists have described many functions of these lipoids, 'their major function, or at least other functions, remains to be discovered' (Dawson, 1957). Most of the work on phospholipines has been done by biochemists. Histochemical work has been very little. Hane (1912), Abe (1924), Konopacka (1924), Froboese (1926), Derman (1926), Hibbard (1928) and Marza (1929) were some of the histochemists who studied phosphlipines in the embryonic stages of certain animals. Later work deals with only certain tissues of adult animals. In the present study eggs of trout were fixed every day to give a compact series of developmental stages. This fish was chosen only as a matter of convenience because its eggs can be fertilised in large number at a time and they can be easily reared in laboratory. In this text EEB stands for extra-embryonic blastoderm.

TECHNIQUE

The material was mostly fixed in formaldehyde-calcium (Baker, 1944) and cut into frozen sections. Routine fixatives were also used. Sudan IV and sudan black B were used as lipoid stains. Acid-haematin test with pyridine-extraction control was used to detect phospholipines. To see lipoids and proteins together in the same sections, they were first stained with Millon's reagent and then were counterstained with sudan black B. Details of the techniques and their limitations and controls have already been published elsewhere (Krishna, 1950).

OBSERVATIONS

For the sake of convenience, the lipoids in the yolk and in the embryo cells have been studied separately; and the incubation period is divided in four stages :

First stage : From the time of fertilisation to 12 or 13 days after it when the EEB has covered the entire yolk.

Second stage : Up to 32 or 33 days after fertilisation when the larva is fully formed and is ready to hatch.

Third stage : Up to about three weeks after hatching.

Fourth stage : Up to the time when the entire yolk sac has been absorbed.

Phospholipines in the yolk :

First stage :—In the early period of development there was a general richness of lipoids in the yolk. The frozen sections were stained with sudan black B and Millon's reagent. The result was a dirty blue colour. The blue colour was dominating. This shows the excess of lipoids. After the acetone treatment when the triglycerides had been removed, the central region of the sections was rich in phospholipines while the surface of the yolk was not so. The stain of sudan black B and acid-haematin was more intense in the centre than on the surface of the yolk. For the detailed study of the phospholipines a section of the egg was divided in three regions as shown in the figure (Fig. 1).

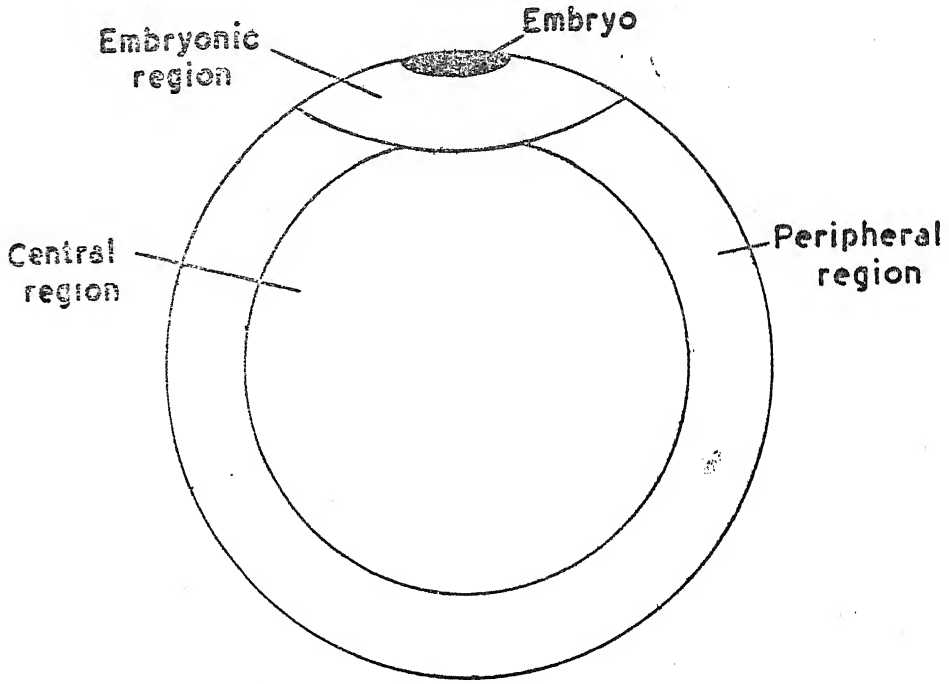

Fig. 1. A sketch showing the parts of a section mentioned in the text.

Central region :—The phospholipines were mainly located here. After acetone treatment the sections had more lipoids in this region than anywhere else. With sudan black B and acid-haematin the stain in the centre was more intense than on the periphery. After the removal of phospholipines in alcohol and ether the quantity of lipoids had very much reduced in this region. Now with sudan black B there was only a light blue colour indicating the presence of lipo-proteins.

Peripheral region :—For this study the peripheral region was divided in two zones : (1) outer zone, which is the surface of the yolk and (2) inner zone, a deeper layer towards the central region. The inner zone did not have any phospholipines. The sudan black B stain was very light in this region showing the presence of lipo-proteins only and the alcohol and ether treatment did not materially affect the quantity of lipoids in this region. Also this zone was homogenous. Unlike it the outer zone (the surface of the yolk) contained droplets formed due to the breaking down of the yolk emulsion. For a few days after fertilisation the outer zone did

not show the presence of any phospholipines. After acetone treatment there were only lipo-proteins left. They took a very light colour with sudan black B and were not stained with sudan IV; and with Millon's reagent and sudan black B the visible colour was red. Later on phospholipines also appeared in this region. The sudan black B and acid-haematin stains were as intense as in the central region. The interesting point was that the part of the outer zone under the EEB showed the presence of phospholipines while the portion outside it was free from them. As the EEB was extending, the phospholipines were appearing below it. When the EEB has completely covered the yolk, the phospholipines were present in the outer zone of the peripheral region and in the central region. The inner zone of the peripheral region was free from them. When a frozen section of this stage, after acetone treatment, was stained with Millon's reagent and sudan black B, it was blue in the central region and in the outer zone of the peripheral region; its inner zone was red showing the absence of phospholipines (Fig. 2).

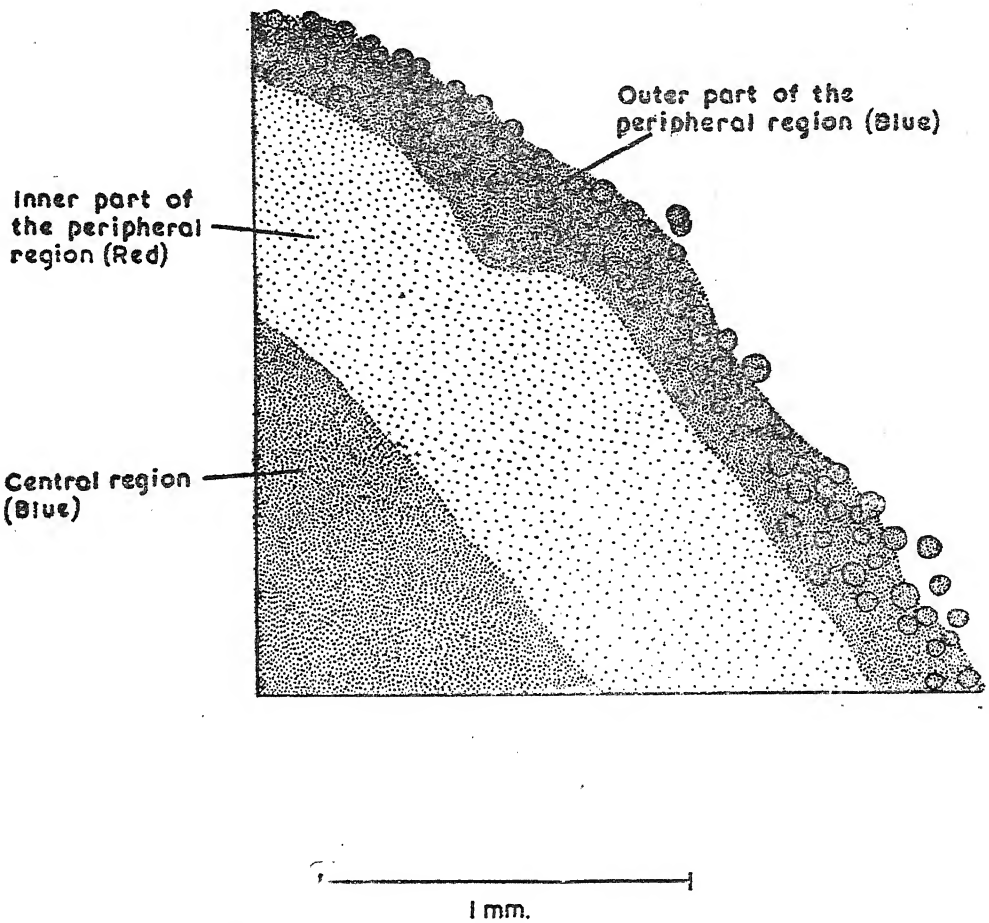

Fig. 2. A part of a frozen section showing phospholipines (stained blue) in the outer part of the peripheral region and the central region, and lipo-proteins (stained red) in the inner part of the peripheral region. Fixed in formaldehyde-calcium treated with acetone and stained with sudan black B and Millon's reagent.

Embryonic region :—In freshly-laid eggs just below the ovum there was a thin strip of phospholipines. It lasted only for a few days after fertilisation. This strip gave positive reactions with all the tests for phospholipines. Following this strip there are lipoid droplets which contain triglycerides and phospholipines. When the phospholipine strip was used up, the lipoid droplets came in contact with the embryo and showed flattening at the points of contact. These droplets did not completely dissolve in acetone. The portion towards the embryo remained undissolved. With nileblue sulphate the droplets were blue. Below these droplets towards the centre of the yolk the phospholipines were absent in the beginning, and the lipoid droplets in this region contained only triglycerides. It was obvious because all the droplets dissolved in acetone leaving no trace behind. But after a few days phospholipines appeared in this region; and then even the lipoid droplets gave positive results with phospholipine tests (Krishna, 1956).

Second stage :—

Central region :—There was no appreciable change.

Peripheral region :—The inner zone was poor in phospholipines. After acetone treatment only lipo-proteins were left there. The outer region was in contact with the EEB. For this region it was the period of vigorous chemical and physical activities. Phospholipines were increasing in quantity and the yolk emulsion was breaking down to form droplets, so much so that the outer zone of the peripheral region consisted mainly of a layer of droplets. Some of the droplets were in intimate contact with the EEB. When a piece of it was picked up with a pair of forceps, there were lipoid droplets on the inner surface of the EEB. These droplets were mainly phospholipine in their composition They were strongly positive to all the tests for phospholipines.

Embryonic region :—It reduced with the growth of the embryo. By the time the EEB was closed, this region was completely taken up by the growing embryo.

Third stage :—During this period there was a considerable decrease in the quantity of triglycerides. Therefore, the phospholipines were in prominence. The control sections at this stage were like the acetone-treated sections of the earlier stages. The double stain of Millon's and sudan black B now gave a dirty blue colour.

Central region :—There was no marked change.

Peripheral region :—No appreciable change in this region too.

Fourth stage :—

Central region :—It had reduced very much.

Peripheral region :—The outer zone was undergoing a rapid change. The phospholipines were becoming prominent and were spreading towards the inner side. As the surface of the outer zone was being used up by the EEB, the inner zone was taking its place.

Phospholipines in the embryo :—

A day after fertilisation, the embryo consisted of only two or four cells. At this stage there were some lipoid bodies with small quantity of phospholipines in

them. The embryo cells continued to multiply. Four to five days after fertilisation the embryo was a bundle of undifferentiated cells. Some of these cells, the fore-runners of endoderm, were in contact with the yolk through the parablastic layer. Lipoids in this layer appeared as fine particles scattered all over. Only nuclei were clear. In a section, stained with sudan black B, the unstained nuclei were showing prominently in the crowd of fine blue particles which covered the entire layer (Fig. 3). These lipoid bodies contained phospholipines. They did not

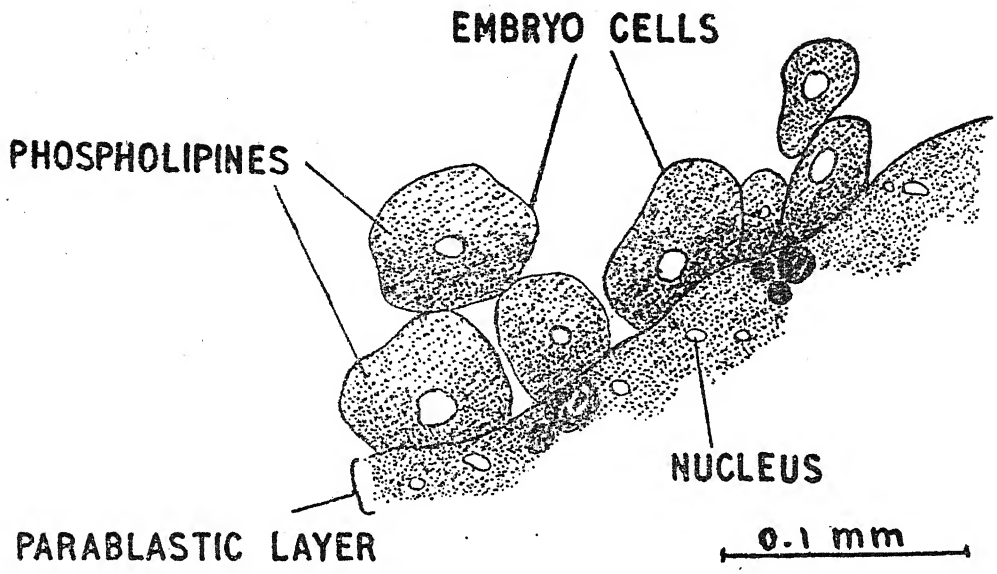

Fig. 3. Camera-lucida showing of a frozen section 4-5 days after festitisation showing phospholipines in the embryo cells more towards the yolk. Formaldehyde-calcium with acid-haematein.

dissolve in acetone, but dissolved in other lipoid solvents. They were positive with acid-haematin. After a treatment with boiling solvents, there was nothing left in the place of the particles.

The lipoid bodies in the cells close to the yolk were fine particles more concentrated towards the yolk than away from it. The lipoid bodies took a blue colour with acid-haematin and sudan black B. Nileblue sulphate stained them blue. It is, therefore, evident that they contained phospholipines. They did not dissolve in acetone, but dissolved in alcohol, ether and other solvents. This treatment washed off the matter stainable with sudan black B, but the position of the lipoid bodies was marked by such bodies which did not stain with lipoid stains. And they were positive with protein tests. In the cells away from yolk the lipoid bodies were

concentrated near the nucleus on one or both the sides of it (Fig. 4). These concentrations of lipoid were very rich in phospholipines. They gave intensely positive reaction to all the tests for phospholipines.

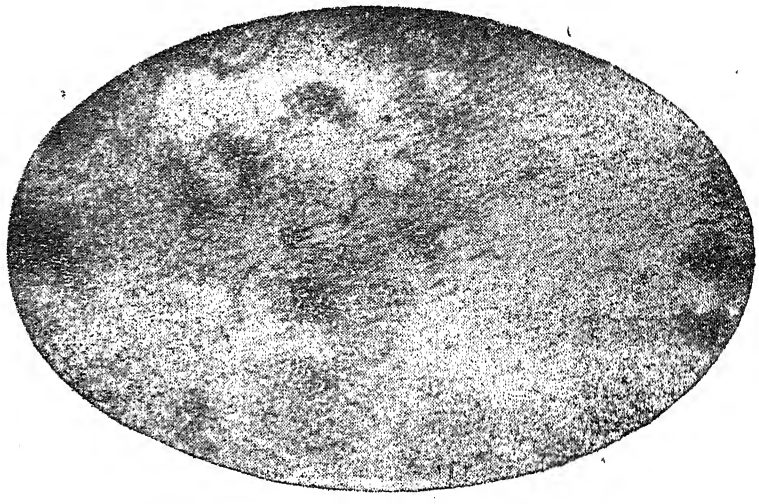

Fig. 4. Photomicrography of a part of a frozen section showing phospholipines in the cells away from yolk Formaldehyde-calcium with acid-haematein X250.

The phospholipines were also present as part of triglyceride droplets contained in the cells. In the next few days (about 12 days after fertilisation) the EEB had covered the yolk, which was now enclosed in a yolk sac formed by the EEB, and its endoderm cells were in contact with the yolk. To begin with the EEB was only two layered - ectoderm and endoderm. Very soon the mesoderm started from the body of the embryo and extended in between the two layers, making the EEB three layered. The endoderm completely encircled the yolk, but the other two layers were absent towards the body of the embryo where the yolk sac was attached to it. When a piece of EEB was picked up and stained, its underneath surface showed very prominent lipoid droplets. These dropets were very rich in phospholipines reacting intensely positive with all the phospholipine tests. Also they completely dissolved in phospholipine solvents, more quickly when they were used warm. Sections of the EEB showed that the endoderm cells were rich in phospholipines. They were present as fine particles scattered all over the cell. The mesoderm and ectoderm cells were very poor in lipoids. Apparently there was no histological or histochemical evidence suggesting a direct relationship between the lipoids in the endoderm cells and those in the mesoderm and ectoderm cells.

INJECTION EXPERIMENTS

As it has been mentioned in the earlier paper of the series (Krishna, 1956) trout embryos are strong enough for experimental work after they have hatched out of the eggs. Therefore the larvae were injected with cod-liver oil, halibut oil, palm-kernel oil, ground nut oil, and trout-egg oil. These were injected into the yolk sacs with and without phospholipines, and their absorption was watched. Trout larvae could take all the oils and none of them produced any toxic effect. The results are given in the table (Table, 1).

TABLE 1

Results of the injection experiments

...	Experiment I Palm-kernel oil		Experiment II Trout-egg oil		Experiment III Ground-nut oil		Experiment IV Ground-nut oil		Condition of yolk-sac in the injected larvae when the control ones had absorbed theirs		
Mixtures injected	No. of larvae used	No. died	No. of larvae used	No. died	No. of larvae used	No. died	No. of larvae used	No. died	when controls had abssorbed the yolk-sac.	15 days after	30 days after
Control (Uninjected) ...	6	0	6	0	6	1(*)	6	0	Absorved		
Oil free or almost free from phospholipines	20	0	17	1	15	0	19	0	Not absorved	Not absorbed	Not absorbed
Oil plus Phospholipines from trout eggs (**)	18	2	21	0	17	1(*)	23	0	Not absorbed	Absorbed	
Oil plus lecithin (B.D.H.) (**)	20	20	21	21	19	19	23	23			
			(In all the experiments the larvae died within a week)								
Oil plus fresh chicken-egg yolk (**)	21	21	18	18	23	23	22	22			
			(The larvae is these experiments died within twenty four hours)								

(*) Denotes accidental deaths. The larvae were caught in the mesh of the tray and died.

(**) Phospholipines were mixed with oils in the proportion of 3:4 (Faure-Fremiet and Garrault, as quoted by Hayes, 1930. Biochem. Journ., 24 : 735).

The results show that the chicken-egg yolk and lecithin (B. D. H.) were not favourable to the larvae. The chicken-egg yolk, because of its richness in phospholipines, was used as a substitue for them. But the phospholipines extracted from the yolk of trout eggs did not produce any adverse effect, and all the injected larvae lived normally. In the injected larvae the yolk sac phase was prolonged by ten days to a fortnight. When the controlled larvae had absorbed their yolk sac, it was still present in the injected ones. A fortnight after the controlled larvae had absorbed their yolk sac, the injected ones were fixed in formaldehyde-calcium and cut into frozen sections. The larvae injected with oil and phospholipines had absorbed the yolk sac completely with its contents, while those injected with oil without phospholipines did not absorb the entire contents of the yolk sac. Certain larvae were allowed to live for another fortnight. Oil was still there. Therefore, utilisation of triglycerides had either completely stopped or was very much slowed down in the absence of phospholipines ; and their presence in triglycerides speeded up the process of utilisation.

DISCUSSION

The study of the location of lipoids shows that the phospholipines have an interesting distribution. In the beginning they are confined to the centre of the yolk. As development proceeds they appear in the region below the embryo and then on the surface of the yolk as the EEB covers it. The space between the surface of the yolk and its centre was always free from phospholipines. This means that the phospholipines on the surface of the yolk have either travelled to this place from

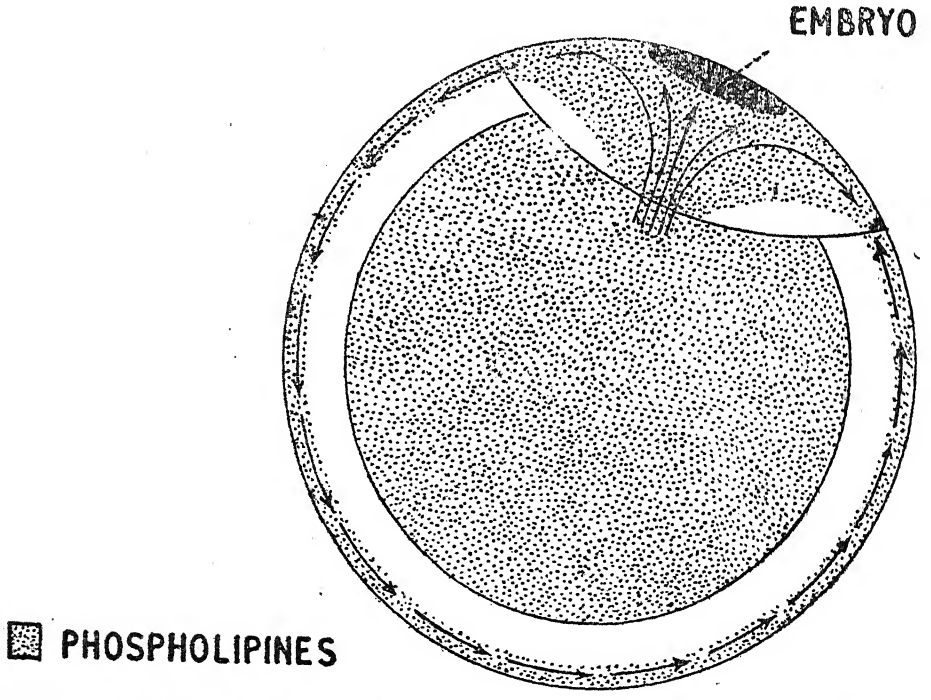

Fig. 5. A sketch showing one possible explanation of how phospholipines appear in the surface of the yolk.

the central region through the embryonic region (Fig. 5) or they have been formed on the surface of the yolk by the breaking down of the lipo-proteins present there.

Moreover, the data also show that phospholipines were not present near the fertilised ovum in the beginning of development. If there were at all any, they were in the form of lipo-proteins. But only a few days after fertilisation large quantities of phospholipines were detected near the growing embryo. When the EEB is closed phospholipines are very prominently present at the points of contact between the embryo cells and the yolk. As the surface of contact between the embryo and the yolk increases phospholipines also spread accordingly. Other lipoids also, which are in contact with the embryo, contain phospholines at this stage. All this indicates an important relationship between phospholipines and metabolism of lipoids. Absence or, to be more correct, deficiency of phospholipines very much slowed down the process of lipoid metabolism. Although histochemical evidence on this point is very scarce, importance of phospholipines in lipoid metabolism has been observed by many biochemists. As early as 1891 Loew suggested that phospholipines were an important component of lipoids during their metabolism. He thought that phospholipines were the carriers of triglycerides and were the intermediate compounds during their matabolism. Since then his thesis has found a wide support among biochemists. Leaths (1909) and Sinclair (1934) have also given their support to Loew's view. Bloor (1943) also supports this theory with a comment that only a certain amount of triglycerides undergo phosphorilation during metabolism. Schluman (1945) has shown the importance of cholin (a base attached to lecithin) during metabolism of triglycerides in mammalian intestines. According to him, in certain cases, phospholipines form a monolayer covering the triglyceride droplet. During metabolism this helps the droplet to pass through the cell wall. Both of these views support the importance of phospholipines during lipoid metabolism.

SUMMARY

(1) Phospholipines have been studied with modern techniques.

(2) They are concentrated in the centre of the yolk, and are also present on its surface. In between these two regions they are absent.

(3) At the points of contact of the yolk and the embryo cells phospholipines are always present.

(4) At certain points phospholipines appeared only when a contact between the embryo cells and the yolk had been established.

(5) It appeared that phospholipines played a very important role in lipoid metabolism. Without the presence of phospholipines either lipoid metabolism stopped or it was very much slowed down. Their presence accelerated the process of metabolism.

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PHYSIOLOGICAL STUDIES ON SALT-TOLERANCE IN CROP PLANTS. X. EFFECT OF NaCl AND Na_2CO_3 ON EARLY SEEDLING GROWTH OF WHEAT AND GRAM*

By

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INTRODUCTION

The depressing effect of sodium salts (NaCl, Na_2CO_3, Na_2SO_4 etc.) on plant growth has been studied in detailed by various workers and the problem has been reviewed by Hayward and Wadleigh (1949) and also by Hayward and Bernstein (1958). The author also worked out in detail the relative tolerance, based on growth and maturity of plants, of two important rabi crops (wheat and gram) and their varieties to NaCl and Na_2CO_3 (Bhardwaj 1958).

Investigating the influence of NaCl and Na_2CO_3 on plant metabolism at the seedling stage (0-96 hours after sowing), it was observed that the crops and also the varieties differ in their relative response to the salts (Bhardwaj 1959a, and Bhardwaj and Rao 1958 and 1960). Since growth is the culmination of metabolic activities, it was planned to find out whether the relative tolerance of the two crops and their varieties to these two salts can be assessed at the early seedling stage itself. It would then facilitate easy and rapid testing of varieties and crops regarding their suitability to saline and alkaline areas.

In the present investigations, an attempt has been made to investigate the influence of supplying NaCl solutions at 0·2% and 0·6% concentrations and Na_2CO_3 at 0·07% and 0·2% concentrations on early seedling growth (till 96 hours after sowing) on two varieties each of wheat (N.P. 165 and C. 591) and gram (N. P. 28 and T. 87). The concentrations of the salts employed here agree with the concentrations found in saline and alkali soils, and were selected on the basis of chemical analysis of these soils by the author (Bhardwaj 1959b) and also reported by soil chemists (Raychaudhri and Tripathi 1953, Sen 1953, and Desai and Sen 1953).

METHOD AND MATERIALS

Influence of NaCl (0·2% and 0·6% solutions) and of Na_2CO_3 (0·07% and 0·2% solutions) have been investigated on early seedling growth (0-96 hours after sowing) of two varieties each of wheat (N. P. 165 and C. 591) and of gram (N. P. 28 and T. 87). Garrard's technique, as modified by Sarin and Rao (1956), for growing the seedlings, was adopted.

Seeds were initially soaked in the test solution, partly immersed, in petri-dishes without filter paper for 12 hours. Two seeds per tube were then transferred to the edge of the filter paper cone and the roots were allowed to grow between the paper roll and the glass wall of the tube. The tubes were kept in an incubator (in darkness) at a temperature of 22°C.

Ten replications (five tubes, each having two seeds) were maintained for each treatment including the controls (distilled water). Observations at 24 hour intervals, upto 96 hours after sowing were recorded for total length of roots and of the plumule. The data were analysed statistically on factorial basis following 'analysis of variance' method.

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* A part of the thesis approved for the Ph. D. degree of the Agra University.

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It may be noted that the seeds began sprouting 24 hours after sowing and thus the root-length could be measured from 48 hours after sowing upto the end of 96 hours ; plumule length was recorded after 72 and 96 hours in wheat and at the end of 96 hours only in gram.

EXPERIMENTAL FINDINGS

The results on the influence of NaCl and Na_2CO_3 on root and plumule growth of wheat and gram and their varieties are repre sented graphically in Figures 1 and 2.

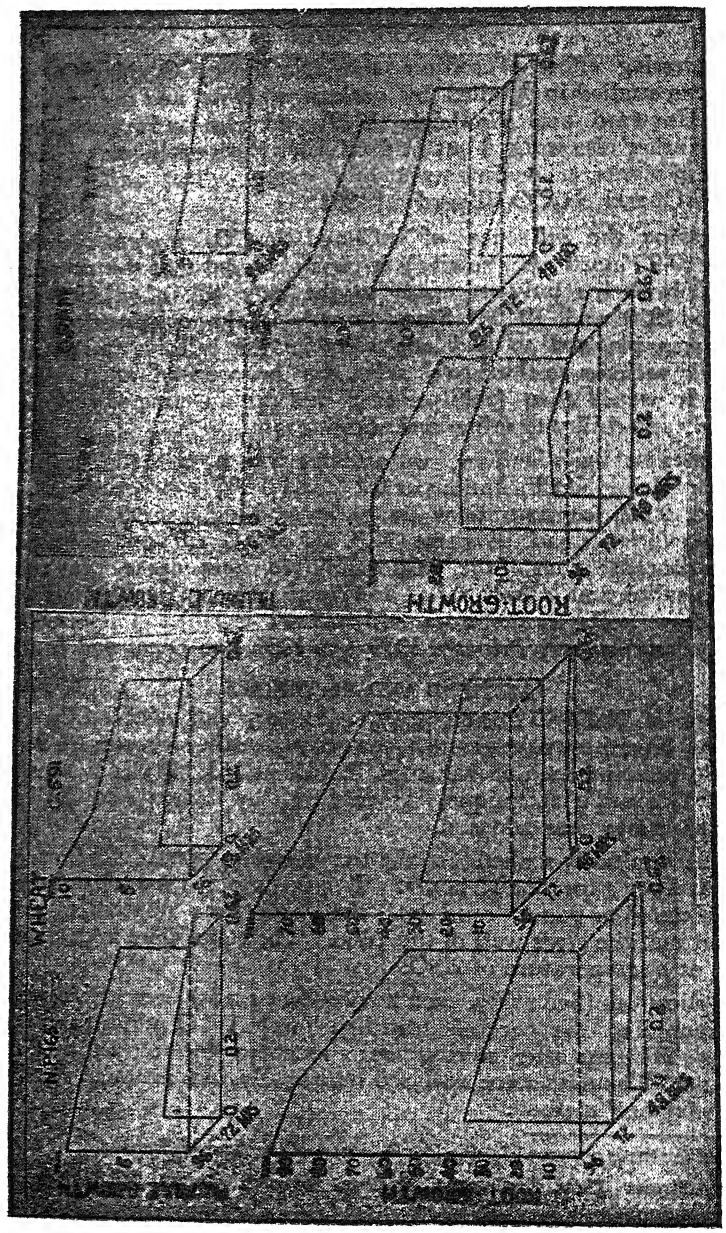

Fig. 1 : Effect of supplying NaCl solution on early seedling growth (till 96 hours after sowing) of wheat and gram varieties.

As the data was analysed statistically on factorial basis (two-factor and three-factor experiments), critical differences at 5% probability for the 'main factors' as well as their 'interactions' are included in Appendix I, wherever the treatments were significant. Also, for a better understanding of the relative response of the

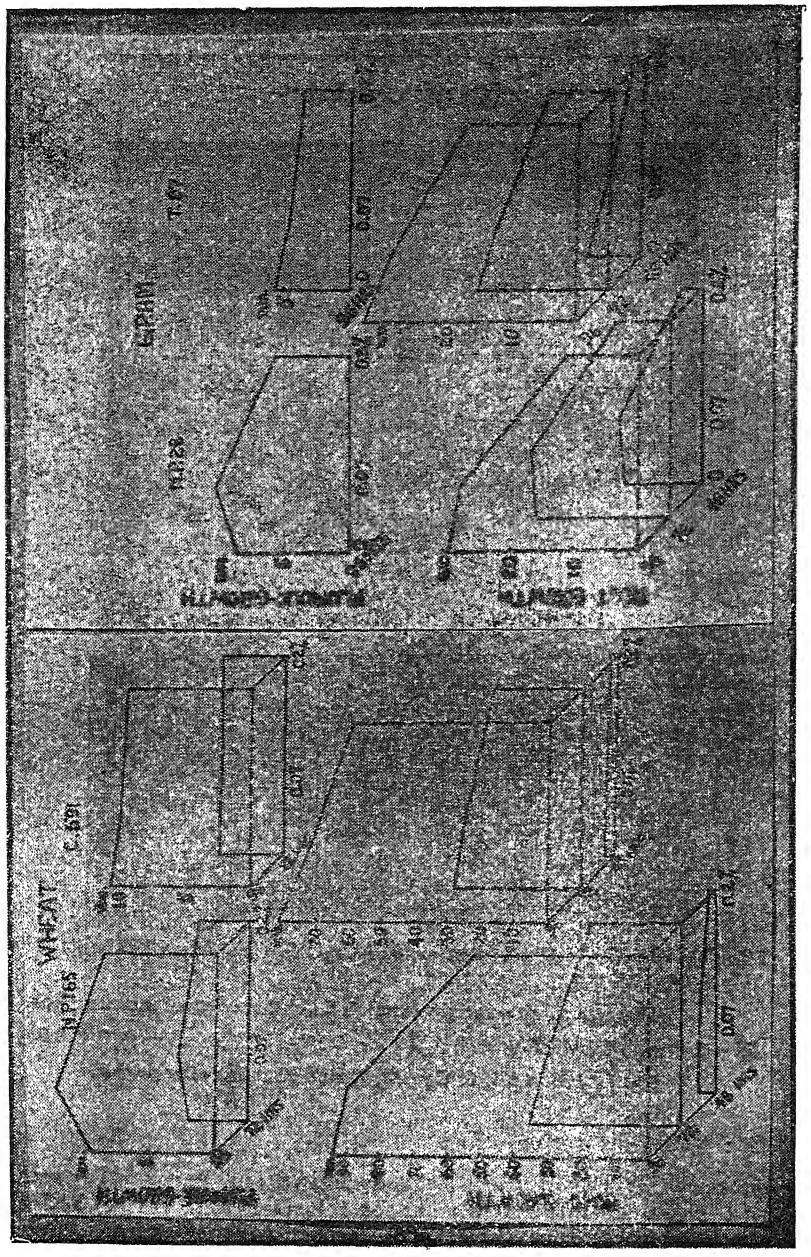

Fig. 2 : Effect of supplying Na_2CO_3 solution on early seedling growth (till 96 hours after sowing) of wheat and gram varieties.

varieties or the crops to the two salts, percentages over respective controls (distilled water supply) have been calculated and are included in Figures 3, 4 and 5.

The results of the two salts are dealt with separately. The average values of the two varieties of each crop are considered for comparing the response of wheat and gram to NaCl and Na_2CO_3.

(*A*) *Effect of NaCl* (*Fig. 3*) :—

The following interesting points are revealed :—

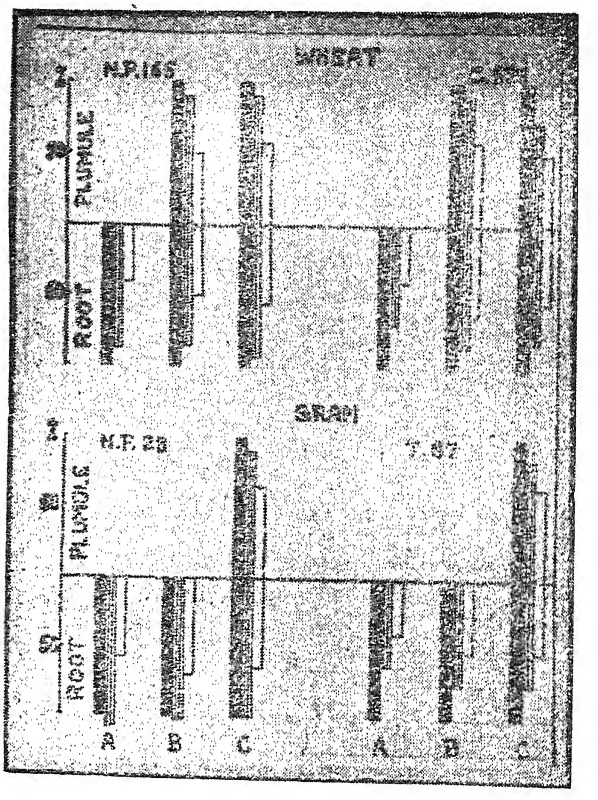

Fig. 3 : Effect of supplying NaCl solution on early seedling growth (0-96 hours) of wheat and gram varieties.

(Percentages on respective controls for each variety of the two crops)

■ : Control ('Distilled water'); ▥ : 0·2% NaCl solution ; □ : 0·6% NaCl solution A B & C : 48, 72 and 96 hours after sowing.

(1) 0·2% NaCl :

(*a*) Root growth : Even the lower concentration apparently retarded the root-growth of the two varieties of wheat; the relatively late variety suffered more; the average reduction in root growth, compared to the controls, for the three periods (48, 72 and 96 hours after sowing) being 11% for N. P. 165 and 21% for

C. 591. Similarly calculated values for the gram varieties indicate that root-growth of relatively early variety N.P. 28 was not affected by the salt supply (0·2%) while the late variety showed a reduction of 30%.

Thus varietal differences in root growth with 0·2% NaCl supply were in general clear in both wheat and gram; the relatively late variety suffered more than the early one.

It is, however, interesting to note that if the averages of the two varieties are taken for comparing the effect of 0·2% NaCl on root growth of the two crops, both wheat and gram responded almost alike, the average reduction in root growth in wheat comes to 16% and that of gram to 13%, compared to their respective controls.

(*b*) Plumule growth: The results show the same trends as observed for the root growth; the early variety suffered less than the late one; differences between wheat and gram are negligible.

(2) 0·6% NaCl:

(*a*) Root growth: Considering the average values for the three period varietal differences in root growth of wheat with 0·6% NaCl supply were negligible; however, in gram varieties, the average percentage reduction in root growth of T. 87 (late variety) was greater than in N. P. 28, the reductions being 52% and 36% respectively.

Comparing wheat and gram, on the basis of the average for the two varieties, percentage reduction in root growth of wheat, with 0·6% NaCl supply, amounted to 49% and of gram to 44%; the difference is negligible.

(*b*) Plumule length: The average values indicate that varietal differences in both wheat and gram were negligible, gram in general seemed to fare better than wheat.

(3) Comparison of wheat and gram:

In general, the higher concentrations retarded the root and plumule growth of both the crops and their varieties to a much greater extent than was noted with 0·2% NaCl. Compared to the respective controls, the root and plumule growth in wheat and gram on an average for the three periods and of the two varieties is indicated below:

Average growth with NaCl-supply
(% on controls)

	Wheat		Gram	
	Root	Plumule	Root	Plumule
0·2%	84	84	87	87
0·6%	51	64	56	64

It is interesting to note that the differences between the two crops are almost negligible with either concentration of NaCl; compared to the controls, the reduction in root and plumule growth amounted to 13-16% with 0·2% NaCl, and to 36-49, with 0·6% NaCl. The slightly better performance of gram cannot perhaps be considered as due to its relatively greater tolerance to NaCl than wheat in the seedling stage.

(*B*) *Effect of* Na_2CO_3 (Fig. 4) :

The noteworth points are :—

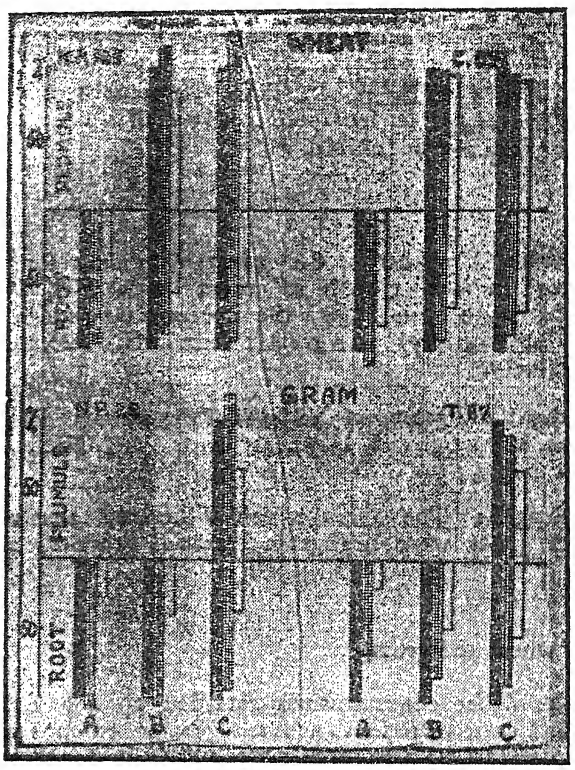

Fig. 4: Effect of supplying Na_2Cl_3 solutions on early seedling growth (0-96 hours) of wheat and gram varieties.

(Percentages on respective controls- for each variety of the two crops)

■ : Control ('Distilled water') ; ▥ : 0·07% Na_2CO_3 solution ; □ : 0·2% Na_2CO_3 solution A, B & C : 48, 72 and 96 hours aftea sowing.

(1) 0·07% Na_2CO_3 :

(*a*) Root growth : The carbonate had practically no effect on the root growth of the two varieties of wheat, the average percentage values (percentage on their respective controls) being 94% for N.P. 165 and 97% for C. 591 ; varietal difference was negligible. In gram, the early variety N.P. 28 was not affected by the carbonate while root growth was definitely reduced in the late variety T. 87 ; the average values are 101% (N. P. 28) and 79% (T. 87).

(*b*) Plumule growth : In wheat, plumule growth in N. P. 165 was definitely improved by this concentration of the carbonate, while there was scarcely any effect on C. 591, the average percentage values being 121% and 96% respectively for the two varieties. Similar to wheat, the early variety of gram (N. P. 28) showed stimulation of plumule growth (119%), while it was depressed in the late variety T. 87 (80%).

(*c*) Comparison of wheat and gram : Comparing the two crops on the basis of the average percentage values of the two varieties (Fig. 5), it is seen :

(*i*) Even with such a low concentration crop differences are indicated ; gram is relatively more susceptible to the carbonate than wheat ; the average values for the mean of the varieties are indicated below :

	Wheat	Gram
Root growth	96%	90%
Plumule growth	109%	100%

(*ii*) Although the early varieties of wheat and gram behaved similarly, the late variety of gram (T. 87) suffered relatively more than that of wheat (C. 591).

(2) 0·2% Na_2CO_3 :

(*a*) Root-growth : In wheat the early variety N. P. 165 was affected more than that of C. 591 the percentage values being 49% and 75%. The varietal differences in gram were practically negligible.

(*b*) Plumule growth : Similar to root growth, plumule growth in the early variety of wheat was affected more than in the late one ; but in gram, the varietal differences were not observed.

(*iii*) Comparison of wheat and gram : With a supply of 0·2% Na_2CO_3 gram definitely suffered more than wheat ; the values for the mean of the varieties are indicated below :

	Wheat	Gram
Root growth	62%	38%
Plumule growth	90%	65%

Further, it was observed that with the lower (0·07%) concentration of the carbonate, the adverse effect on root growth of wheat and gram varieties (except gram T. 87) gradually increases with time (vide Fig. 4 values for 48, 72 and 96 hours after sowing). With the higher concentration (0·2%), there is gradually an initial large retardation of root growth at 48 hours, particularly of the early variety of wheat (N. P. 165) and of gram (N. P. 28) ; it is followed by a gradual and continuous recovery or recovery limited to the following 24 hours (i.e. upto 72 hours).

(C) *Comparison between NaCl and Na_2CO_3* (Fig. 5) :

Attempt has been made to understand the relative influence of NaCl and Na_2CO_3 on the root and plumule growth of the two crops. The two salts resulted in depressed growth of roots and of plumule of wheat and gram, particularly by the higher concentration (0·06% NaCl and 0·2% Na_2CO_3). Depression in root growth by either salt was maximum at 48 hours after sowing after which a tendency to recover was obvious.

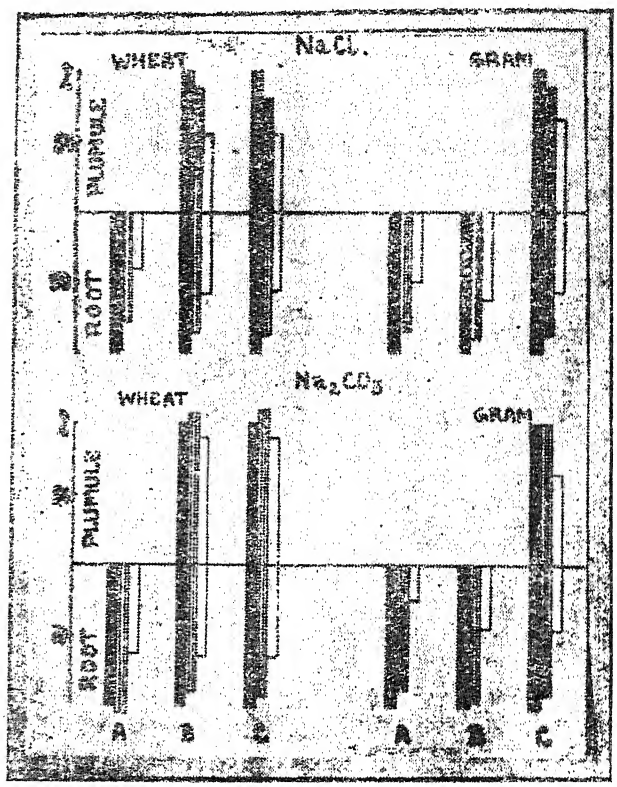

Fig. 5 : Effect of supplying NaCl and Na_2CO_3 solutions on early seedling growth (0-96 hours) of wheat and gram (mean of varieties).

(Percentages on respective controls- for each salt and crop separately)

■ : Control ('Distilled water') ; ▥ : 0·2% NaCl or 0·07% Na_2CO_3 solution ; □ : 0·6% NaCl or 0·2% Na_2CO_3 solution.

A B & C : 48, 72 and 96 hours after sowing.

Further interesting features are :

(i) With a supply of NaCl solution (0·2% or 0·6%) wheat and gram seem to behave almost similarly regarding root or plumule growth, upto 96 hours after sowing, as indicated by the average percentage values. The adverse effect was more intense with 0·6% NaCl. Varietal differences in wheat are negligible ; in gram the late variety (T. 87) seems to fare better than the early one (N. P. 28).

(*ii*) With a supply of Na_2CO_3 solution (0·07%), the effect on seedling growth of wheat and gram was slight. Plumule growth seems to be stimulated in the early variety of wheat or gram. With the higher concentration (0·2%), crop differences are clear ; gram suffered more. Varietal differences are not clear. In general, gram seems to be affected more than wheat.

DISCUSSION

Literature relating to the effect of NaCl or Na_2CO_3 on seedling growth is apparently lacking. In general, crop or varietal differences to these salts, based on growth and maturity, are mentioned by several workers. Legumes are generally known to be more susceptible than creals, a conclusion which is indicated by the present results on seedling growth. However, in this laboratory Sarin and Rao (1956) have studied the influence of Na_2SO_4 on early seedling growth (upto 4 days after sowing) of wheat (C. 591) and gram (N.P. 58), and observed that the salt resulted in depressing the growth of roots as well as of plumule, gram suffering more than wheat.

It is worthwhile to compare the results obtained with NaCl in the present study with those of Sarin and Rao (1956). The following figures in Table I give a comparative idea of the effect of Na_2SO_4 and NaCl, supplied at the same concentrations, on seedling growth of wheat and gram.

TABLE I

Effect of Na_2SO_4 and NaCl on seedling growth (till 96 hours after sowing) of wheat and gram. (Growth with salt-supply at the end of 96 hours as percentage on respective control).

	Na_2SO_4		NaCl	
	0·2%	0·6%	0·2%	0·6%
Root growth:—				
Wheat	90	70	88	56
Gram	64	42	88	58
Plumule growth:—				
Wheat	96	76	80	54
Gram	67	49	87	64

Note : (*i*) The data for Na_2SO_4 are taken from the results of Sarin and Rao (1956); the results relate to wheat C. 591 and gram N. P. 58 and the seedlings were grown at 30°C.

(*ii*) The data for NaCl are the average for the two varieties of wheat and of gram in the present study; the seedlings were grown at 22°C.

Although the results for the two salts are not strictly comparable due to variations in the conditions of the two experiments, the differential response of

wheat and gram to NaCl or to Na_2SO_4 even at the early seedling stage needs further study.

As already pointed out the aim of the present study was chiefly to investigate differential response, if any, of varieties and crops to NaCl or to Na_2CO_3 even at their early seedling stage. If these responses are confirmed by similar behaviour of the varieties and crops at later stages of growth and maturity, testing of varieties or crops regarding their salt or alkali tolerance will be considerably facilitated. It may be pointed out that the object of the study is not fully achieved, as the varietal or crop responses noted by the author, based on growth and maturity of the plants, are not fully confirmed, although indications for the crop differences observed at maturity stage are fairly clear even here.

It is thought that time-factor is quite important in assessing the effect of the salt on the early seedling growth and on later growth and maturity. The accumulation of the salt or its ions in the cells is perhaps much less than in the case of fully grown plant, continuously fed with salt. Further, the seedling metabolism cannot be idenfical or even similar to the metabolic activities of the full-grown green plant. Even so, the effect of the salts on seedling metabolism is sufficiently clear and may even differ with the crops, as observed by Bhardwaj and Rao (1958 and 1960). Crop or varietal differences (particularly the latter) in growth of the seedling, the resultant of the metabolic activities, have not, however, revealed themselves sufficiently clear within the short duration of four days from sowing. The promising results indicated here and on seedling metabolism (Bhardwaj & Rao 1958 and 1960) are sufficiently encouraging to undertake further detailed investigations on seedling metabolism and growth of varieties and crops on a comparative basis.

Theoretical considerations on salt or alkali effect :—

It is not intended to discuss the various aspects of salt or alkali injury on the basis of the present data which relates only to seedling growth. However, as growth is the culmination of metabolic activities, any indication of 'injury', as seen by retardation of growth, can give some approximate idea for the mode of action of the salt.

A careful scrutiny of the progress of growth of roots and of plumule with NaCl or Na_2CO_3 supply reveals :—

(*i*) With the lower concentration (0·2%) of NaCl which barely gives an osmotic pressure of 1·3 atmospheres, the influence on growth seems to be due to the intrinsic effect of the accumulating ions, Na^+ or Cl^-, on the living cells; stimulation or toxicity depends evidently on the concentration of the ion, particularly Cl^-; crop or varietal differences, particularly the former may become evident, even parts of the seedlings, roots, or plumules, in the early stage, may differ; with lapse of time leading to accumulation of ions, the depressing effect becomes clear even in the plumule growth which was stimulated earlier.

(*ii*) With a supply of the higher concentration of NaCl (0·6%), which is definitely hypertonic in relation to the lower dose, the primary or initial effect on root growth seems to be osmotic. The initial depressing effect on root growth soon disappears at 72 hours after sowing, but is sometimes followed by relapsed depression. Plumule growth is less affected initially (72 hours for wheat) and the adverse effect increases with time (at 96 hours), the recovery could not be noted as the experiment stopped at 96 hours after sowing.

The initial check on root growth is perhaps due to the osmotic effect; the recovery can be due to the increase in the concentration of the cell sap by accumulation of the soluble ions; the later relapse to inhibition of growth can be due to the toxic effect of the ions. Further, the initial osmotic effect does not seem to vary with the crop or even with the variety. The later toxic effect may vary with the crop or variety.

(*iii*) With a supply of sodium carbonate, the osmotic effect does not seem to exist as the higher dose is only 0·2% concentration. The effect is mainly toxic and differs with the crops and the varieties. As in all cases of toxic substances, in general, very small doses may stimulate as noted for plumule growth of wheat and gram with a supply of 0·07% Na_2CO_3.

Finally it may be noted that the above discussion is strictly hypothetical, as data on osmotic pressure of the tissues and on chemical analysis of the same for accumulating ions are lacking. These studies are now intended to be taken up for detailed investigation.

SUMMARY

Following modified Garrard's techniques (Sarin and Rao 1956), seedlings of wheat (N. P. 165 and C. 591) and of gram (N. P. 28 and T. 87) were grown in darkness at 22°C. Total length of roots and of plumule were measured daily upto 96 hours. The influence of supplying solutions in two concentrations each of NaCl (0·2% & 0·6%) and of Na_2CO_3 (0·07% and 0·2%) on the early seedling growth of wheat and gram varieties was studied.

The conclusions based on the above studies are :—

(*i*) *NaCl* :—Wheat : Both the concentrations of the salt depressed the growth of roots and of plumule in the two varieties; the effect was more pronounced in the higher concentrations. The differences between the varieties were small but, in general, N. P. 165 fared better than C. 591, particularly regarding the plumule growth at the end of 96 hours.

Gram : Growth of roots and of plumule in N. P. 28 was inhibited only slightly by 0·2% NaCl solution, compared to the more pronounced effect on T. 87. The higher concentration was deterimental to both the varieties; apparently N. P. 28 fared better than T. 87.

(*ii*) Na_2CO_3 :—Wheat : The lower concentration (0·07% solution) showed little adverse effect on growth of roots and plumule of the two varieties; it even stimulated plumule growth in N. P. 165. In the higher concentration seedling growth in the two varieties was depressed, plumule growth to a less extent than root growth. C. 591 fared apparently better than N. P. 165.

Gram : The higher concentration (0·2% solution) was definitely detrimental to the seedling growth of both the varieties ; the varietal differences were small and apparently in favour of T. 87. However, the lower concentration depressed the seedling growth of T. 87 alone, while it even stimulated plumule growth in N. P. 28.

(*iii*) Seedling growth (upto 96 hours after sowing) in the two crops, wheat and gram, suffered almost similarly by a supply of NaCl solution; plumule growth in gram was, however, slightly better in wheat. With Na_2CO_3 supply, it is clear that gram was more susceptible than wheat.

ACKNOWLEDGEMENTS

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APPENDIX I

Effect of supplying NaCl and Na_2CO_3 solutions on early seedling growth (till 96 hours after sowing) of wheat and gram varieties.
(C. D. at 5% level in mm., whenerer the expt. is significant)
Salt–supply.

	NaCl				Na_2CO_3			
	Wheat		Gram		Wheat		Gram	
	Root growth	Plumule growth	Root growth	Plumule growth	Root growth	Plumule growth	Root growth	Plumule growth
Main factors :–								
(*i*) Variety	4·5	0·2	3·3	1·3	–	–	–	1·5
(*ii*) Salt-effect	5·5	0·3	4·0	1·6	6·1	–	2·5	1·9
(*iii*) Age	5·5	0·2	4·0	×	6·1	1·0	2·5	×
Interaction :–								
(*i*) × (*ii*)	–	0·4	5·5	–	–	–	3·5	–
(*i*) × (*iii*)	–	–	–	×	–	–	3·5	×
(*ii*) × (*iii*)	3·5	0·4	–	×	10·6	–	4·3	×
(*i*) × (*ii*) × (*iii*)	–	0·5	–	×	–	–	–	×

STUDIES ON THE NUTRITION OF FUNGI

V. THE INFLUENCE OF THE VARIOUS CARBON SOURCES ON THE GROWTH OF *ALTERNARIA BRASSICAE* (BERK.) SACC

By

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The first and the third papers in the series give an account of the influence of different sources of carbon on the growth of *Colletotrichum capsici* (Thind and Randhawa, 1957) and *Gloeosporium psidii*, *G. piperatum* and *Colletotrichum* sp. (Thind and Rawla, 1958) respectively. This paper deals with the influence of carbon sources on the growth of *Alternaria brassicae*.

MATERIAL AND METHODS

Alternaria brassicae was isolated from *Brassica campestris* and its several monosporic cultures showed similar characteristics and all produced abundant spores. One of these isolates was selected and maintained on P. D. A. (potato 200 gms., dextrose 20 gms., agar 20 gms. and distilled water 1,000 mls.) for further experimentation. Rest of the procedure was similar to that mentioned in Papers I and III on the series. The basal medium (dextrose 15 gms., KNO_3 5 gms., KH_2PO_4 5 gms., $MgSO_4 . 7H_2O$ 0·5 gms., $Fe_2 (SO_4)_3 . 6H_2O$ 0·001 gm. and distilled water 1,000 mls.) was found to be suitable for the growth of *A. brassicae*. However, while studying the effect of various carbon compounds dextrose was excluded from the basal medium and it was replaced by each carbon compound* singly so as to provide the same amount of carbon as is present in 15 gm. of dextrose. Fifty millilitres of the media were taken in a 250 ml. Erlenmeyer flask. Three replicates were taken in each case. The media were seeded by the addition of 1 ml. standardized spore suspension (10 spores per field of the low power of microscope) to each flask and incubated for 6 days at 28°C. Six days incubation period, 28°C temperature and pH 4·5 of the medium were found to be the optimum conditions for the growth of this pathogen by preliminary investigation. Determinations of the dry weight of the mycelial growth and final pH were made as usual.

EXPERIMENTAL WORK

As many as 40 carbon compounds, comprising 14 carbohydrates, 14 carboxylic acids, 7 alcohols, 3 oils, 1 ketone and 1 amide were used singly as sole sources of carbon in order to observe their effect on the growth of this fungus. The basal medium (excluding dextrose) was taken and sterilized at 15 lb. pressure for 15 minutes. The various carbon compounds were dissolved separately in distilled water and the pH was adjusted to the neutral point to minimize the

* Starch, Inulin and Pectin were added at the rate of 15 gms. per litre. The three oils were added at the rate of 15 mls. per litre.

TABLE I

Average dry weight and final pH of *A. brassicae* on different carbon sources after 6 days incubation at 28°C, initial pH adjusted to 4·5.

Carbon source	Average dry weight in milligrams	Average final pH
Control (without carbon)	0	4·5
d (+) xylose	131	6·3
l (+) arabinose	98	5·8
Dextrose	298	5·9
Fructose	99	5·8
d (+) mannose	106	5·4
d (+) galactose	39	4·9
Sucrose	301	7·1
Lactose	48	5·4
Maltose	255	6·4
Melezitose	83	5·8
Raffinose	102	6·3
Starch	158	6·1
Inulin	116	
Pectin	271	7·0
Formic acid	0	4·5
Acetic acid	0	4·5
Propionic acid	0	4·5
Butyric acid	0	4·6
n-Valeric acid	0	4·6
Lactic acid	13	4·7
Glycollic acid	0	4·4
Pyruvic acid	85	5·8
Oxalic acid	13	4·4
Maleic acid	41	5·1
Succinic acid	184	4·8
Sebacic acid	0	4·5
Tartaric acid	0	4·5
Citric acid	90	4·4
Mannitol	127	6·3
Dulcitol	25	4·6
Glycerol	34	5·5
Methyl alcohol	25	4·9
Ethyl alcohol	15	4·7
Isopropyl alcohol	0	4·5
n-Butyl alcohol	27	5·4
Castor oil	17	4·9
Cotton seed oil	23	5·3
Olive oil	24	5·8
Acetamide	12	6·5
Acetone	15	6·6

possibility of hydrolysis during autoclaving. Sterilzation of carbon compounds was done at 10 lb. pressure for 10 minutes. These compounds were then added separately and aseptically to the remainder of the medium and initial pH adjusted to 4·5.

EXPERIMENTAL RESULTS

The results presented in Table I indicate that sucrose, dextrose, pectin and maltose yielded excellent growth (300-255 mgs.); succinic acid good growth (184 mgs.); while starch, xylose, mannitol, inulin, mannose, raffinose, fructose, arabinose, pyruvic acid and melezitose fairly good growth (158-83 mgs.). Rest of the carbon compounds either did not support any growth or it was very poor. Growth of the fungus shifts the pH towards neutrality in general.

DISCUSSION

Excellent growth of *A. brassicae* on sucrose, dextrose and maltose is, in general, in agreement with other fungi, although Tandon and Grewal (1954), reported only fairly good growth of *A. tenuis* on these sugars. Similarly Pawar and Patel (1957), recorded best growth of *A. ricini* on maltose and dextrose but poor growth on sucrose. *A. brassicae* is unique in exhibiting excellent growth on pectin, which does not seem to have been reported for any other fungus so far. On the other extreme, *A. brassicae* exhibited very poor growth on galactose. In this respect it resembles *Aspergillus niger* (Steinberg, 1939), *Blastocladia* spp. (Craseman, 1957) and *Colletotrichum* sp. (Thind and Rawla, 1958). However, Tandon and Grewal (1954), Thind and Sandu (1956), and Pawar and Patel (1957), noted that galactose is a fairly good substitute for glucose in the case of *A. tenuis*, *Gloeosporium psidii* and *A. ricini* respectively. In its poor growth on lactose, it resembles *Colletotrichum capsici* (Thind and Randhawa, 1957) and three anthracnose fungi (Thind and Rawla, 1958). However, Tandon and Grewal (1954) and Pawar and Patel (1957), recorded fairly good growth of *A. tenuis* and *A. ricini* respectively on lactose. *A. brassicae* gave fairly good growth on xylose, arabinose, mannose, starch and inulin, whereas *A. tenuis* is recorded to give poor growth on xylose, arabinose and inulin but fairly good growth on mannose and starch (Tandon and Grewal, 1954). Pawar and Patel (1957), recorded best growth of *A. ricini* on xylose, inulin and starch but poor growth on arabinose.

Failure of *A. brassicae* to utilize tartaric acid is very interesting because it is reported to be utilized by most of the fungi. In this respect it resembles *Blastocladia* spp. (Craseman, 1957). Good growth of this pathogen on succinic acid is also interesting because it usually supports poor to fairly good growth with fungi.

A. brassicae gave fairly good growth on mannitol but very poor on dulcitol and glycerol. However, Tandon and Grewal (1954), recorded best growth of *A. tenuis* on all these alcohols, while Thind and Rawla (1958), observed fairly good growth of *G. psidii* and poor growth of *G. piperatum* and *Colletotrichum* sp. on mannitol and dulcitol. Similarly Thind and Randhawa (1957), and Pawar and Patel (1957), recorded poor growth of *C. capsici* and *A. ricini* respectively on these two alcohols.

SUMMARY

Forty different carbohydrates were tested singly as sole sources of carbon to find out their influence on the growth of *A. brassicae* isolated from *Brassica campestris*. This study was carried out at 28°C for 6 days and initial pH of the medium was

adjusted to 4·5 in each case. Sucrose, dextrose, pectin and maltose yielded excellent growth; succinic acid good growth; while starch, xylose, mannitol, inulin, mannose, raffinose, fructose, arabinose, pyruvic acid and melezitose fairly good growth. Rest of the carbon compounds either did not support any growth or it was very poor.

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PHYSIOLOGICAL STUDIES ON SALT-TOLERANCE IN CROP PLANTS. XI. INDUCING TOLERANCE TO NaCl BY PRE-TREATMENT OF SEEDS

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INTRODUCTION

In arid and semi-arid regions of Northern and Central India, vast areas of land lie barren owing to high salinity, sodium chloride being the predominating salt. Some soil ameliorative measures like green manuring and cultivation of paddy were adopted in the past to reclaim such areas. (Mann and Tamhane 1910, Inglis 1927, Inglis and Gokhale 1928, Talati 1947). The problem of improving plant growth in areas with low and moderate salinity has generally been overlooked, possibly because no external symptoms of injury are noticeable and the poor growth is taken to be due to low fertility of the soil. As Eaton (1942) pointed out that a substantial proportion of the curtailed production of crops in irrigated areas that was attributed to nutritional deficiencies or unfavourable water relations was in fact due to saline conditions customarily regarded as insufficiently high to be a cause of reduced yields.

During the last six years, a series of experiments were conducted in the Department of Botany, Agra College, Agra to assess the losses incurred due to soil salinity (Bhardwaj 1960), to investigate its effect on early seedling metabolism and growth of the plants growing in such areas (Bhardwaj 1961) ; and Bhardwaj and Rao 1958 and 1960), to find out the tolerant crops and their varieties (Bhardwaj 1958); and to explore the possibility if tolerance could be induced by pretreatment of seeds (Bhardwaj 1958).

The present paper deals with the investigations made to induce tolerance in the crop plants, wheat and gram, to NaCl by presowing soaking of the seeds in dilute solutions of NaCl, and later on growing them in soil containing a harmful dose of NaCl. Their growth and maturity were compared with those of plants raised in similar soil but from untreated seeds and from seeds soaked in water before sowing.

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One set of plants was also subjected to soil-drought conditions for four weeks during February (13th to 16th week after sowing). The treatment of soil-drought during February was introduced to simulate the adverse conditions obtained during rabi season in this locality when plants experience adverse weather conditions in the form of warm dry winds and the field soil becomes dry so that in saline areas the concentration of the salts in the soil solution may increase considerably.

METHODS AND MATERIALS

Grain of wheat (C. 591) and seeds of gram (N. P. 28) were soaked, partly immersed, in solutions of sodium chloride (0 and 1000 ppm) in petri-dishes without filter paper for a period of 24 hours either continuously or intermittently, the latter comprising of three cycles of 8 hours soaking followed by 16 hours drying in shade in atmosphere with relative humidity of 80 – 90 percent, and were immediately sown in earthern pots (9″ × 9″), each filled with 4·0 kilograms of air-dry soil mixed with garden compost. Untreated seeds (control) were sown a day earlier. NaCl at 0·1% concentration (on air-dry soil) was added to the respective pots in water solution soon after sowing the seeds. 10 seeds per pot were sown but four weeks after sowing, the seedlings were thinned to four during rabi 1953-54 and 1954-55 and to three during rabi 1955-56 per pot. Necessary precautions were taken to collect the percolating soil solution from the pots and to add it back to the same.

When plants were maturing (13th to 16th week after sowing) one set was subjected to permanent wilting till the mositure percentage of the soil reached about 6%; thus soil-moisture in these pots varied from field capacity (about 25%) to 6%, while optimum water supply (from about 25% to 17%) was maintained throughout in another set.

Observations on growth, as indicated by the dry weight of the tops, yield of grain (or seed) and weight of 100 seeds were recorded. The results were analysed statistically according to analysis of variance, on factorial basis. The critical difference at 5% level is tabulated along with the 'means' where the treatments were significant.

EXPERIMENTAL FINDINGS

Influence of pre-sowing seed-treatments on growth and yield of the two crops in soil containing 0·1% NaCl has been dealt separately and the results are included in Tables 1 and 2 for wheat and gram respectively.

A. *Wheat C.* 591 :—

The experiment was conducted for three consecutive cropping seasons (rabi 1953-54, 1954-55 and 1955-56), and the results of each season are discussed separately.

TABLE 1

Influence of presowing seed-treatments on growth and yield of wheat plants grown in soil containing 0·1 per cent (on air dry soil) sodium chloride.

A. *Main factors* (1 : Presowing seed-treatment ; 2 : Soil—moisture)

Observation	Control	Presowing soaking in NaCl solution				C. D. +	Soil-water-supply		C. D.
		Cntinuous*		Intermittent**			Optimum @	Soil droughts @@	
		0-ppm	1000-ppm	0-ppm	1000-ppm				
Season : 1953-54 :—									
Shoot dry weight (g) ...	1·34	1·12	1·21	1·08	1·02	0·18	1·50	0·80	0·14
Grain yield (g) ...	0·95	0·98	1·11	1·05	1·02	...	1·25	0·79	0·13
100-grain weight (g) ...	3·86	3·90	4·13	4·20	3·77	0·06	4·31	3·63	0·05
Season : 1954-55 :—									
Shoot-dry wt. (gm) ...	0·96	1·79	2·27	1·02	1·18	0·33	1·75	1·14	0·21
Grain yield (gm) ...	0·58	0·94	1·53	0·85	0·85	0·22	1·39	0·51	0·14
100-gr. wt. (gm) ..	2·52	2·84	3·29	3·35	3·51	0·23	3 58	2·63	0·14
Season : 1955-56 :—									
Shoot-dry-wt. (gm) ...	1·88	1·61	1·37	2·28	1·97	0·28	2·00	1·64	0·18
Grain yield (gm) ...	0·72	0·72	0·55	1·03	0·81	0·16	1·13	0·41	0·11
100 gr. wt. (gm) ...	1·73	3·01	2·65	3·13	2·56	0·37	4·18	1·45	0·24

TABLE 1—(*concld.*)

B Interaction (Presowing seed-treatment × soil moisture)

Observation	Soil—Water Supply										
	Optimum@					Soil-droughts@@					
	Control	Presowing soaking in NaCl solution				Control	Presowing soaking in NaCl solution				C. D.+
		Continuous*		Intermittent**			Continuous		Intermittent		
		0-ppm	1000-ppm	0-ppm	1000-ppm		0-ppm	1000-ppm	0-ppm	1000-ppm	
	Season : 1953-54 :—										
Shoot dry weight (g)	1·75	1·46	1·48	1·52	1·31	0·94	0·78	0·95	0·64	0·72	...
Gr. Yield (gm) ...	1·05	1·16	1·40	1·49	1·16	0·85	0·81	0·82	0·61	0·88	0·22
100-gr. wt. (gm) ...	4·17	4·18	4·53	4·73	3·95	3·56	3·62	3·75	3·67	3·59	...
	Season : 1954-55 :—										
Shoot-dry wt. (gm)	1·14	2·00	3·03	1·18	1·39	0·78	1·57	1·51	0·85	0·97	0·47
Gr. Yield (gm) ...	0·85	1·29	2·34	1·19	1·27	0·31	0·58	0·71	0·51	0·43	0·99
100-gr. wt. (gm) ...	3·33	3·17	3·62	3·73	4·01	1·71	2·51	2·96	2·98	2·98	0·31
	Season : 1955-56 :—										
Shoot-dry wt. (gm)	1·92	1·93	1·49	2·46	2·21	1·84	1·28	1·25	3·09	1·72	...
Gr. Yield (gm) ...	0·98	0·88	0·83	1·51	1·44	0·46	0·56	0·27	0·56	0·18	0·24
100-gr. wt. (gm) ...	3·91	3·23	4·13	4·34	4·27	1·56	1·78	1·15	1·82	0·85	0·53

* Continuous : Continuous soaking for 24 hours.

** Intermittent : 3 Cycles of 8 hours soaking followed by 16 hours drying at RDH 80—90 %.

@ Optimum : Water supply optimum throughout.

@@ Soil-droughts : Subjected to the influence of soil-droughts from 13th to 16th weeks after sowing.

+ C. D. : Critical difference at 6 % probabitity.

(a) *Results of* 1953-54 *Experiments* :

Shoot-dry-weight : Dry matter produced by the shoots (minus grain) at harvest was influenced by the soaking treatments as well as soil-moisture. Reductions in the yield of straw were noted with soaking in water (continuous or intermittent) or in 1000 ppm NaCl solution (intermittent alone), and no improvement was observed in the remaining treatments; soil droughts, irrespective of soaking treatments, lowered the dry matter produced considerably (Table 1A.)

Yield and 100-grain weight : With optimum water supply an increase in yield was noted with soaking either in water (intermittent) or in 1000 ppm NaCl solution (continuous), while on subjection to soil-droughts no such increase was indicated (Table 1 B.)

100-grain-weight was significantly improved, irrespective of soil-droughts, with soaking in water (intermittent) or in 1000 ppm NaCl solution (continuous); soil-droughts, however, lowered the 100-grain-weight considerably (Table 1A).

(b) *Results of* 1954-55 *Experiments* :

Shoot-dry-weight : Under optimum water supply and also soil-droughts, dry matter produced by the shoots was higher by continuous soaking in water or 1000 ppm NaCl solution.

Yield and 100-grain weight: Under optimum water supply, all the seed-treatments resulted in increased yield of plants, the order being :

$$\overline{\text{CSN}} \quad \underline{\text{CSW}} \quad \overline{\text{ISN}} \quad \underline{\text{ISW}} \quad \underline{\text{US}}$$

(CSN : Continuous soaking in NaCl solution ;
CSW : Continuous soaking in water ;
ISN : Intermittent soaking in NaCl ;
ISW : Intermittent soaking in water ; and
US : No soaking (control).)

Under subjection to soil-droughts, the yield per plant was also improved by the seed-treatments, but the order of superiority was slightly altered ; the order being :

$$\overline{\text{CSN}} \quad \underline{\text{CSW}} \quad \overline{\text{ISW}} \quad \underline{\text{ISN}} \quad \underline{\text{US}}$$

The quality of grain, as determined by 100-grain-weight, was superior to control by all the seed-treatments (except continuous soaking in water).

Under optimum watering, 100 grain weight was higher by soaking in 1000 ppm NaCl solution than in water, especially when soaking was continuous. Under soil-droughts, it was higher by all the seed-treatments as compared to its control and the order of superiority was ISN=ISW=CSN>CSW>US. It may be further added that grains produced by the plants subjected to soil droughts,

irrespective of seed-treatment, were of inferior quality than those produced by the plants grown under optimum water supply throughout.

(C) *Results of 1955-56 Experiment* :

Shoot-dry-weight : Dry matter (minus grain) produced by the shoots, irrespective of seed-treatment, was reduced by the soil-droughts (Table 1A). Continuous soaking in 1000 ppm NaCl solution resulted in decrease of straw while intermittent soaking increased the same, and the remaining seed-treatments indicated no response (Table 1A).

Yield and 100-grain weight : Under optimum water supply yield per plant was improved by intermittent soaking in either water or 1000 ppm of NaCl solution while under soil-droughts grain yield was erratic and no improvement over its control was observed ; on the contrary, intermittant soaking in 1000 ppm NaCl solution lowered the yield considerably (Table 1B).

No significant increase in 100-grain-weight was observed by any of the seed-treatments under conditions of optimum water-supply ; however, trends for improvement were obvious in all of them. Under soil droughts as well, no improvement was evident ; intermittent soaking in 1000 ppm NaCl solution resulted in lowering of the same (Table 1B).

B. *Gram N. P. 28* :

Here the experiment was carried out during one cropping season (rabi 1954-55), and the results are given in Table 2.

Shoot-dry-weight : Total dry matter produced by the shoots remained unaffected under optimum water supply, but it was improved slightly by intermittent or continuous soaking in 1000 ppm NaCl solution when plants were subjected to the influence of soil-droughts.

Yield and 100-seed weight : Under optimum water supply neither the yield of seed nor its 100-seed weight was improved by any of the seed-treatments; on the contrary, lowered yields were obtained by continuous soaking in water or NaCl solution or intermittent soaking in NaCl solution alone; 100-seed-weight was reduced by intermittent soaking in NaCl solution alone. Under soil-droughts intermittent soaking in water increased the yield of seeds, while 100-seed-weight was improved by continuous soaking in NaCl solution.

DISCUSSION

Following the homeopathic principle "set a thief after a thief", an attempt was made to aclimatize the seed-embryo to saline conditions and thus induce tolerence to salinity (NaCl) by pre-sowing soaking of seeds. The results on growth and yield of plants, expressed as percentage on respective controls, are briefly recapitulated before discussing their significance under 'General consideration'; and are represented graphically in figures 1 and 2 for wheat and gram respectively.

TABLE 2

Influence of presowing seed-treatment on growth and yield of gram plants grown in soil containing 0·1% (on air dry soil) sodium chloride.

A. Main factors (1 : Presowing seed-treatment ; 2 Soil-moisture.)

Observation	Control	Presowing soaking in NaCl solution				C.D+	Soil-water-supply		
		Continuous*		Intermittent**					
		0-ppm	1000-ppm	0-ppm	1000-ppm		Optimum @	Soil-droughts @	C. D
Shoot dry weight (g) ...	1·15	1·14	1·33	1·10	1·27	...	1·44	0·37	0·21
Seed yield (g) ...	0·73	0·53	0·47	0·81	0·56	0·22	0·90	0·34	0·14
100-seed weight (g) ...	10·5	11·0	11·6	10·6	8·9	1·5	12·1	8·9	1·0

B. Interaction (Presowing seed-treatment × soil-moisture)

Observation	Control	Soil—Water—Supply									C. D.
		Optimum				Control	Soil—droughts				
		Presowing soaking in NaCl solution					Presowing soaking in NaCl solution				
		Continuous		Intermittent			Continuous		Intermittent		
		0-ppm	1000-ppm	0-ppm	1000-ppm		0-ppm	1000-ppm	0-ppm	1000-ppm	
Shoot-dry weight (g)	1·67	1·26	1·44	1·45	1·39	0·63	1·02	1·21	0·76	1·16	0·43
Seed yield (g) ...	1·30	0·74	0·63	1·11	0·74	0·16	0·35	0·32	0·52	0·38	0·32
100-seed weight (g) ...	13·3	13·2	12·5	12·4	9·3	7·7	8·8	10·8	0·87	8·6	2·2

(A) *Wheat* :—

The effect of the seed-treatments was not quite consistent in the three years (Fig 1). Considering the mean values for the three years, as also the results in each year, the following observations can be made :—

(*i*) With optimum water supply, the seed-treatments gave definitely better yields of grain than their respective controls (no-seed treatment) ; intermittent soaking of seeds in water or in NaCl solution was better than continuous soaking. Soaking of seeds in salt solution was more beneficial than in water, when soaking was continuous; the difference between the two was negligible under intermittent soaking conditions.

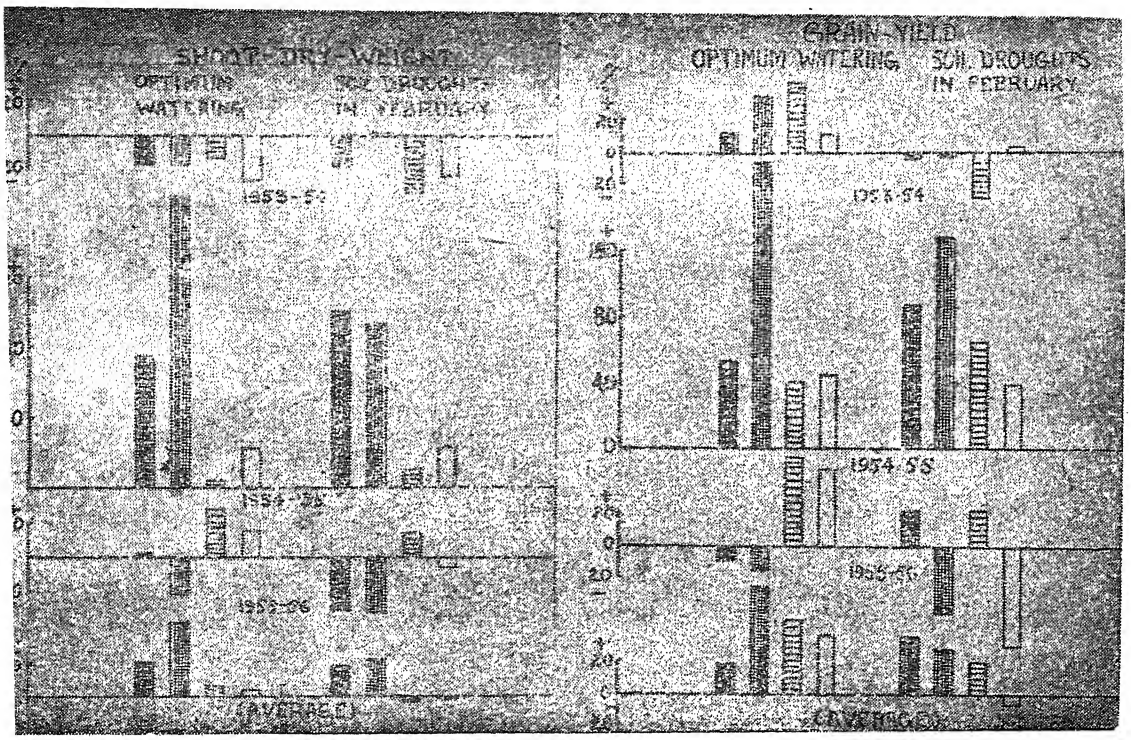

FIG. 1 : Influence of presowing seed-treatments on shoot dry-weight at harvest and grain yield of wheat C.591 grown in soil containing 0·1% (on air-dry basis) NaCl. (Percentage on respective control—'Seeds untreated').

CSW : : 'Continuous' presowing soaking of seeds for 24 hours in distilled water :

CSN : : 'Continuous presowing soaking of seeds for 24 hours in 1000 ppm. of NaCl solution ;

ISW : : Intermittent' presowing soaking of seeds for 24 hours in distilled water ; and

ISN : : 'Intermittent' presowing soaking of seeds for 24 hours in 1000 ppm of NaCl solution.

(*ii*) With soil-droughts during February, the seed-treatments in general gave slightly better yields than the plants from untreated seeds : but, unlike under optimum water supply, continuous soaking was apparently more helpful than intermittent soaking. The differences due to soaking in NaCl solution or in water, both for continuous and intermittent types, were not consistent in the three years.

(B) *Gram* :

The noteworthy features made out (fig. 2) are :

(*i*) With optimum water supply, the seed-treatments in general depressed considerably the yield of seed compared to control.

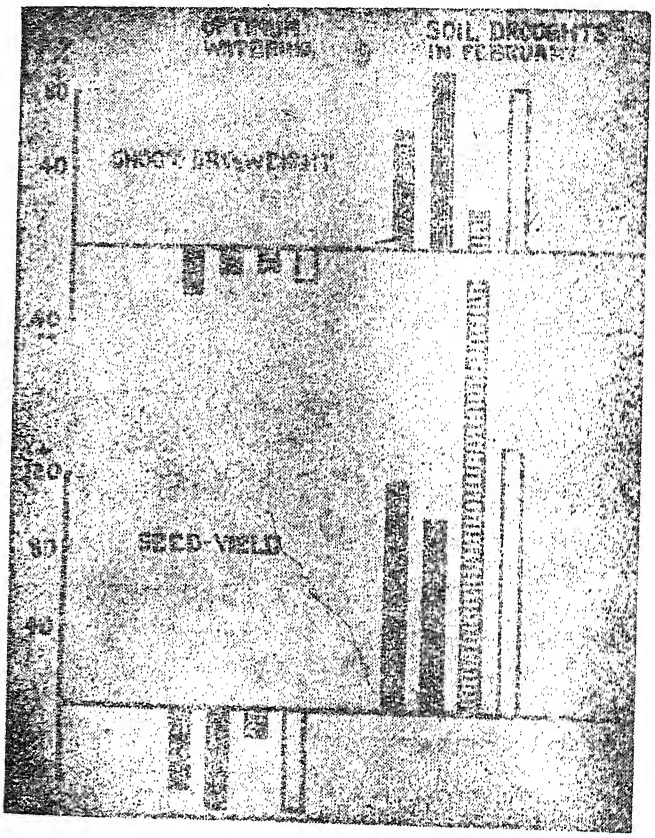

FIG. 2 : Influence of presowing seed-treatments on shoot dry-weight at harvest and grain yield of gram N. P. 28 grown in soil containing 0·1% (on air-dry basis) of NaCl. (Percentage on respective control—'Seeds untradated.').

CSW : : 'Continuous' presowing soaking of seeds for 24 hours in distilled water;

CSN : : 'Continuous' presowing soaking of seeds for 24 hours in 1000 ppm. of NaNl solution;

ISW : : 'Intermittent' presowing soaking of seeds for 24 hours in distilled water ; and

ISN : : 'Intermittent' presowing soaking of seeds for 24 hours in 1000 ppm of NaCl solution.

(*ii*) With soil-droughts in February, which coinsided with the maturity of gram, it showed favourable response to the seed-treatment, both regarding yield of shoot-dry-matter and of seed.

(C) *Comparison between crops*: A comparison has been made between the responses exhibited by wheat C. 591 and gram N. P. 28. It is revealed :

(*i*) Under optimum water supply, shoot-dry-weight at maturity and yield of grain in wheat was either improved by some pre-sowing seed-treatments or almost the same as for the control; in gram, the seed-treatments led mostly to depression in the above characters.

(*ii*) With soil-droughts in February, trends in wheat were almost similar to those obtained under optimum water supply; in gram too, improvement in dry matter production and yield of seed by the seed-treatments was indicated.

(*iii*) Thus under the conditions of the present study, most of the pre-sowing seed-treatments did improve growth and yield of wheat plants raised in saline soils containing (0·1% NaCl on air-dry-basis) under the two levels of soil moisture; but the treatments were effective for gram only under the soil-drought conditions.

It is noticed that specific seed-treatment were responsible for improvement of growth and yield. 'Intermittent' soaking in water indicated distinct superiority over the rest of soaking treatments under the influence of either level of soil-moisture for the two crops. Thus, it is interesting to note that 'water-soaking' was generally as good, if not better than 'soaking in NaCl solution'.

General Considerations. According to Kidd and West (1918), physiologic predetermination occurs in germinating seeds, apparently initiated by the genetic constitution of embryo. This predetermination decides, in general, the form and development of the growing plant. During germination there is intensive metabolic activity involving the mobilization of the stored food, its transport to the growing embryo and its utilization in the formation of new cells. Thus at such highly dynamic or plastic stage of the embryo, it may get easier adapted to adverse environmental conditions and the acquired adaptation may persist till the maturity of the plant. On such an assumption, apparently, several workers attempted to induce tolerance in the plants to drought, frost, etc., by pretreatment of seeds.

Resistance to drought was observed by pre-sowing soaking the seeds in NaCl solution, preferably alternate soaking and drying, in rice plants by Parija (1943) and Parija and Pillay (1945) and in wheat by Chinoy (1942 and 1947). In Russia, Henkel and Kolotova (1940) made studies regarding salt-resistance of wheat ; seeds were soaked in 1 M solution (5·85%) of NaCl for one hour and the dried seeds were sown in the field, the treated plants had greater salt-resistance.

Thus, it seems clear that pre-sowing soaking treatment of seeds affects the growth was maturity of the plants; its nature (adverse or beneficial effect), intensity and duration depends on the type of the treatment itself.

With soaking the seeds in water alone, it has been noted that temperature, oxygen supply and carbon-dioxide concentration during the period of treatment can considerably influence the response; soaking in distilled water is generally found to be harmful, due to the leaching of the salts, proteins, or even enzymes and vitamins

from the germinating seeds (Crocker and Barton 1953). However, in the present studies where 'soaking the distilled water' (continuous or intermittent) was included as one of the treatment, the results are varied; often growth and maturity were even better than the control (seed untreated) but occurrence of adverse or no effect was also common. Soaking in NaCl solution' has not generally proved more beneficial than 'soaking in water'. Again the mode of soaking, continuous or intermittents, has not given consistent results

Thus it become necessary to evalute the conditions, both during the treatment period as well as later on, which will lead to beneficial effects always.

SUMMARY

Influence of pre-sowing soaking seed-treatment has been investigated in wheat and gram with a view to inducing tolerance to sodium chloride. Seed treatments to induce tolerance to NaCl tried are:

Control ('seed untreated'); 'continuous' soaking for 24 hours is water (distilled) and in 1000 ppm of NaCl solution; and 'intermittent' soaking for 24 hours in water and in 1000 ppm of NaCl solution.

The soaking technique involved keeping the seeds half-immersed in the test solution in petridishes without filter paper. The seeds were sown in soil (in pots) to which 0·1% NaCl (on air-dry basis) was added thus making the soil moderately saline. The influence of the seed-treatments was studied both under optimum conditions of water-supply throughout (soil-moisture varying from field capacity to about 17%) and under soil-droughts (soil-moisture varying from field capacity to about 6%) during February.

Tentative conclusions based on the above investigation are:

Compared to control, presowing soaking of wheat seeds in water or in salt solution results in better growth and maturity of plants even when the soil is saline, provided the soil-water-content is favourable throughout the life cycle of plants; but if the plants experience soil-droughts during their earning period (February) the effect of the treatments is less favourable and also inconsistent in different cropping seasons. Gram, however, seems to respond favourably to seed-treatments only under conditions of soil-droughts during February; with favourable water supply the seed-treatments are definitely harmful to gram.

ACKNOWLEDGEMENTS

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A NEW GALL MIDGE (ITONIDIDAE : DIPTERA) FROM INDIA

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Family Itonodidae

Subfamily Itonididinae

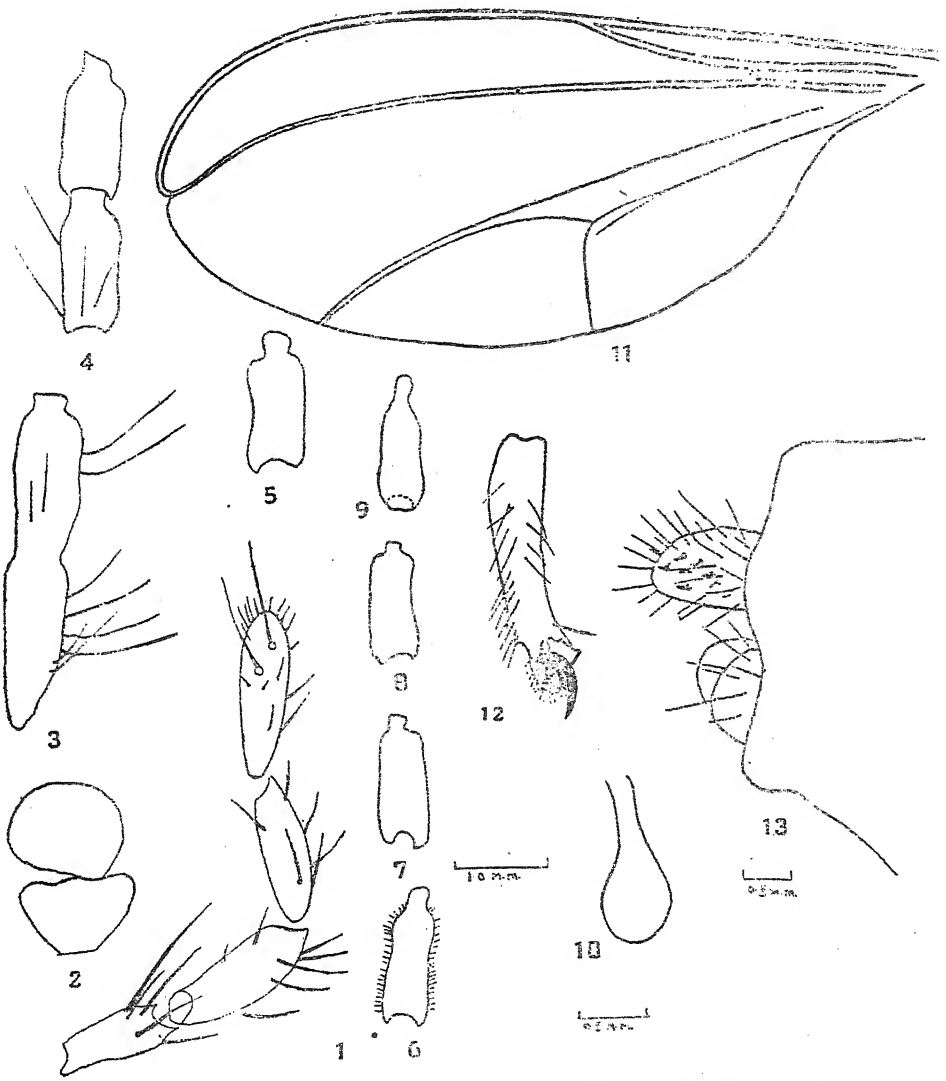
Tribe Porricondylini

The species described below is the fifth under this genus. *Vanchdiplosis* Nayar (1949) was erected in 1949 with *V. vanchii* Nayar as the type. Rao (1950, 1960) added three more species under this genus.

Vanchidiplosis kamali, sp. nov.

Female.—1·54 mm. long, pale-brown. Eyes confluent above. Trophi produced; neck prominent. Palpi (Fig. 1) quardriarticulate, long, sparsely setose, setae long; first segment dark brown, cylindrical, short, length two and three-fourth of the maximum thickness in the middle; second segment longer than the first, broad subapically, length three and one-third times its maximum thickness; third segment shorter than the second, and longer than the first, cylindrical, length four-and-a-half times its maximum thickness; fourth segment cylindrical longer than the third and the second, wider apically than the base, length four and one-fifth times its maximum thickness. Antenna dark brown, with 15 segments, less than half the length of body, segments with one whorl of setae at the base of enlargements, with short apical stems and gradually becoming shorter and slender towards the apex; scape (Fig. 2) yellowish brown, wider apically than the base, wider than long; pedicel (Fig. 2) a little longer than the scape, globose and apically dark brown; third segment (Fig. 3) fused with fourth, as long as first and second segments combined, enlargement with short stem at the base, stem a little less than one-sixth the length of segment and one and one-third of its maximum thickness, enlargement nearly three-fourth the length of segment and two and five-seventh times as long as broad, apical stem very short; fourth segment (Fig. 3) nearly equal to the third, enlargement a little less than the length of segment and nearly two and a-half times as long as broad, stem one-ninth of the segment and one-half as long as broad; fifth sement (Fig. 4) a little shorter than the fourth, enlargement nearly two and one-fourth times as long as broad, stem nearly one-fifth the length of enlargement and wider than long; sixth segment (Fig. 4) a little shorter than fifth, enlargement five-sixth of the segment and a little over twice as long as broad, stem one-fifth of enlargement, three-fourth as long as broad; seventh segment (Fig. 5) as long as fifth, enlargement a little shorter and stem a little longer than that of the fifth segment, stem as long as broad; tenth segment (Fig. 6) nearly similar to seventh segment, a little narrower than the latter; eleventh segment a little shorter than the tenth, enlargement two-and-a-half times as long as broad, stem nearly one-seventh of enlargement and two-third as long as broad; thirteenth segment (Fig. 7) similar to eleventh in all respects

except the stem, stem as long as broad ; penultimate segment (Fig. 8) slightly shorter than the thirteenth segment, enlargement similar to that of the latter segment, stem one-fifteenth the length of segment and one-half as long as broad ; terminal segment (Fig. 9) as long as tenth and longer than the penultimate segment, enlargement with apical teat-like projection, a little less than one-fifth the length of segment and twice as long as broad, enlargement a little less than thrice its maximum thickness. Mesonotum dark brown ; scutellum and post scutellum lighter ; abdomen light brown. Halteres pale-yellow (Fig. 10) Wings (Fig. 11) neither too long nor too broad, hyaline, length a little less than thrice the breadth, with four long veins, vein R_s distinct and making an angle with vein R_1, costa and



Figs. (1) Palpus. (2) Scape and pedicel. (3) Third and fourth antennal segments. (4) Fifth and sixth antennal segments. (5) Seventh antennal segment. (6) Tenth antennal segment. (7) Thirteenth antennal segment. (8) Penultimate segment. (9) Terminal antennal segment. (10) Halter. (11) Wing. (12) Hind claw. 13. Ovipositor

vein R_I covered with hair, Vein R_I uniting with costa before the middle of wing, vein M_{I+2} present, apical half very distinct than the basal half, vein R_5 curved and reaching the wing margin well beyond the wing apex, vein C_u forked. Legs long, blackish brown, densely hairy, metatarsus short, a little less than one-eighth the length of second tarsal segment, shorter than the terminal tarsal segment, second tarsal segment longest of all, nearly as long as the following segments combined, third segment half the length of second, fourth segment a little less than one-third the length of second segment, terminal tarsal segment one-sixth the second segment and one-third of the third segment, longer than the metatarsus; claw (Fig. 12) dentate, bifid basally, dark brown, evenly curved, empodium nearly half the length of claw; ovipositor (Fig. 13) small, slightly exserted, pale-brown, with oval terminal lobes, sparsely setose.

Holotype.—One female dissected and mounted on slide labelled, "at light, Dak bungalow, P. G. coll, Mahijam, Bihar, July 1960."

This species is easily separated from the known species by the different proportion of the palpal and antennal segments and legs and also in the wing venation, pale brown ovipositor with oval lobes, also differs from that of the known species. Its position among other known species is shown below:

KEY TO SPECIES

1. Terminal palpal segment spatulate, longer than the third; empodium nearly equal to claw; lobes of ovipositor oval ... *vanchii* Nayar

   Terminal palpal segment cylindrical; empodium half or one-fourth the length of claw ... 2.

2. Terminal palpal segment nearly equal to the third; empodium half the length of claw; lobes of ovipositor triangular ... *agraensis* Rao

   Terminal palpal segment longer than the third; lobes of ovipositor oval ... 3.

3. Antenna half the length of body; third and fourth antennal segments equal; second palpal segment shorter than the fourth; empodium less than half the length of claw ... *brevipalpi* Rao

   Antenna half the length of body, second and third palpal segments equal or unequal ... 4.

4. Third antennal segment longer than the fourth ; second and third palpal segments equal in length ; empodium half the length of claw ... *longipalpi* Rao

Third antennal segment nearly equal to fourth ; second palpal segment longer than third, shorter than fourth ; empodium nearly half the length of claw ... *kamali* sp. nov.

I am greatly indebted to Prof. M. D. L. Srivastava, Head of the Zoology Department, Allahabad University, for facilities. The author expresses her deep gratitude to Dr. S. N. Rao for constant help. She wishes to record her thanks that are also due to the Government of India for the award of a Junior Research Scholarship, and she is also grateful to Dr. S. N. Prasad for constant supervision, guidance and encouragement.

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INTER-GENERIC VARIATIONS IN FREE AMINO-ACID CONSTITUENTS OF THE ADULTS OF *LAEMOPHLOEUS MINUTUS* OLIVIER AND *ORYZAEPHILUS SURINAMENSIS* LINNAEUS (CUCUJIDAE : COLEOPTERA)

By

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[Received on 20th August, 1960]

INTRODUCTION

Laemophloeus minutus Olivier and *Oryzaephilus surinamensis* Linnaeus the two important pests of milled products, belong to the same family and have similar food habits. The object of the present work is to know the effect of biological grouping and food habits upon amino-acid constituents.

MATERIAL AND TECHNIQUE

The Flat grain beetle and the Saw toothed grain beetle adults were starved for 48 hours after which 100 mgm. of each species were thoroughly rinsed with double distilled (glass) water to remove extraneous matter and were ground with a pinch of anhydrous sodium sulphate and 95% ethanol. The homogenates thus prepared were centrifuged three times at 3000 R. P. M. for 10 minutes every time. The supernatants were reduced to 0·4 ml. The descending paper chromatographic technique of Consden, Gordon and Martin (1944) was followed as was done earlier in the case of similar studies with *Anthrenus vorax* (Chatterji and Sarup, 1960). There were three replications for each experiment and they were repeated six times in the successful solvent mixture.

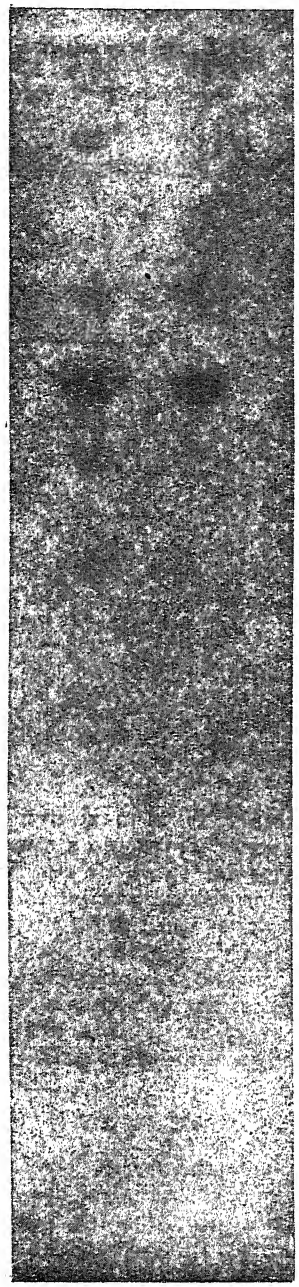
Sheets of Whatman filter paper No. 1 were lined and spotted with the materials as well as standards of known amino-acids. In each case 100 λ of concentrated supernatant was spotted in order to keep the weight of the insects and the reduced volume of supernatants at one particular level.

Different combinations of *n*-butanol, glacial acetic acid and water were tried and the best results were obtained by the solvent mixture having *n*-butanol, glacial acetic acid and water in the ratio of 4:1:5 (v/v/v) (Slotta & Primosigh, 1951). In order to have the best separation, the chromatograms were run four times in the same solvent mixture. After each run they were dried for about 4 to 6 hours and after the last run for 24 hours. The chromatograms were sprayed with 0·1% ethanolic ninhydrin solution (Block *et al.*, 1955). After spraying the dried chromatograms were kept in dark for 24 hours and later developed in 10 minutes in an electric oven at 85°—90°C. The spots were identified by comparing the R_f values of unknown spots with the R_f values of the spots of known materials. In addition to the standards of the known amino-acids which were run side by side with the unknown materials, a separate filter paper blank was also run in the same solvent mixture and sprayed with ninhydrin solution to check up if any spot developed due to any impurity in the filter paper. The investigations were carried out at 90°-94°F. except where mentioned otherwise.

OBSERVATIONS AND DISCUSSION

Fourteen spots of free amino-acids were developed on the chromatogram (Plate 1 of *Laemophloeus minutus* and *Oryzaephilus surinamensis* adults by the technique

PLATE 1



L. minutus *O. surinamensis*

mentioned above. They have been identified as lysine, histidine, arginine, asparagine, serine, glycine, threonine, alanine, proline, methionine, tyrosine, tryptophan, valine and leucine group (leucine, isoleucine, norleucine). The quantitative estimation of these amino acids as judged by the colour density is indicated in the following table.

Amino-acid	*L. minutus* adults	*O. surinamensis* adults
Leucine group ...	++	+
Valine ...	++	+
Tryptophan ...	+	*t*
Tyrosine ...	+	*t*
Methionine ...	+	*t*
Proline ...	+++	+++
Alanine ...	++++	++++
Threonine ...	+++	++
Glycine ...	++	+
Serine ...	++	+
Asparagine ...	+	*t*
Arginine ...	++	*t*
Histidine ...	+	*t*
Lysine ...	+	*t*

'*t*' = in traces

This table shows that alanine is in maximum quantity in the adults of both the species and proline comes next. Tryptophan, tyrosine, methionine, asparagine, arginine, histidine and lysine appear to be present only in traces in *O surinamensis* adults. On the whole, except for proline and alanine, the colour densities of all other spots in *O. surinamensis* adults were lighter than those of *L. minutus* adults. This indicates that the quantitative requirements of these amino acids in *O. surinamensis* are less than those of *L. minutus*, another member of the same family. It is difficult to account for this difference as both have similar food habits and belong to the same group also. However, there does not appear to be any inter-generic qualitative difference in the free amino-acid constituents.

SUMMARY

Lysine, histidine, arginine, asparagine, serine, glycine, threonine, alanine, proline, methionine, tyrosine, tryptophan, valine and leucine group amino-acids have been detected as body constituents of adults of *Laemophloeus minutus* and *Oryzaephilus surinamensis*. Certain inter-generic differences observed are the presence of seven out of fourteen amino-acids only in traces in *Oryzaephilus surinamensis* indicating that

the quantitative requirements of these amino-acids in *Oryzaephilus surinamensis* are comparatively less than those of *Laemophloeus minutus*.

ACKNOWLEDGMENT

The authors are thankful to Dr. E. S. Narayanan, Head of the Division of Entomology, Indian Agricultural Research Institute, New Delhi, for his keen interest in these investigations.

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UTILIZATION OF A MIXTURE OF SUGARS BY SOME SAPROLEGNIALES

By

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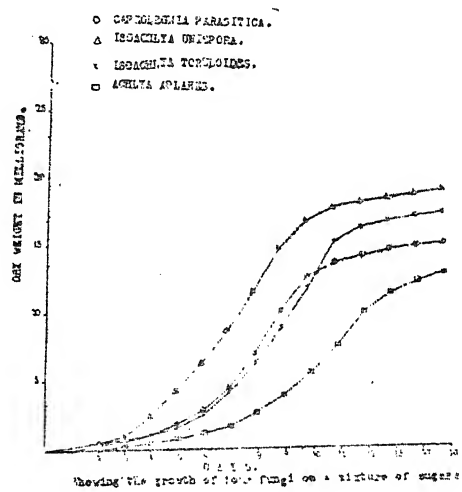
[Received on July 4, 1960]

INTRODUCTION

Fungi growing in nature come into contact with mixed sources of carbon. Some of the common carbon sources occurring in nature include, arabinose, xylose, glucose, fructose, mannose, galactose, sucrose, cellulose, starch etc. Many authors are of the view that fungi grow better on a mixture of carbon sources than on individual ones.

Horr (1936) has investigated the effect of glucose and galactose in a mixture on the growth of *Aspergillus niger*. After several calculations the author found that the yield on the mixture of glucose and galactose was much more what it should have been if these two sugars were utilized independently without one affecting the utilization of the other. Steinberg (1939) working on *Aspergillus niger* reported that some combinations of poor carbon sources resulted in an increased growth of the fungus as compared to when they were used individually. On the other hand, the same author found that the mixture of poor carbon sources also resulted in a decreased growth of some fungi. According to Margolin (1942) the effect of mixed carbon sources on the growth of *Phycomyces blakesleeanus* and *Pythiomorpha-gonapodyoides* were totally additive.



showing the growth of four fungi on a mixture of sugars

Earlier investigations by the author (Ram Dayal, 1958) had shown that various sugars were not of equal value for the growth of these organisms. It was, therefore, considered desirable to study their influence when they were used in

*KH_2PO_4 0·5 gm., $Mgcl_2 \cdot 6H_2O$ 0·5 gm., Na_2S 0·17 gm., NH_4NO_3 2·0 gm. and double distilled water 1 litre.

a mixture. Five sugars were selected for the study. In this combination two disaccharides, viz., sucrose and lactose were also included, because they do not get hydrolyzed by the present organisms, as seen by the author in a separate experiment.

MATERIALS AND METHODS

The organisms under study, viz., *Achlyo aplanes* Maurizio, *Isoachlya unispora* Coker and Couch, *I. toruloides* Kauffman and Coker and *Saprolegnia parasitiea* Coker were isolated locally from a pond using hemp seeds as baits. Single spore cultures were made according to the technique of Couch (1939). Five sugars namely glucose, fructose, galactose, lactose and sucrose were added to the basal medium* in quantities so as to furnish 2000 mgs. of carbon per litre collectively. The pH of the medium was adjusted to 7·0 by 4% solution of sodium hydroxide before autoclaving. The methods for chromatographic analysis were the same as described by the author (1959). The spray reagent was prepared from aniline-diphenylamine and phosphoric acid (5 vols. of 4% aniline, 5 vols. of 4% diphenylamine (both in N-butanol and 1 vol. of phorphoric acid). The average R. f. values of the various sugars have been calculated on the basis of the bands developed.

EXPERIMENTAL

The results obtained for various fungi have been recorded in Table 1. The R. f. values of the different sugars calculated are as follows: Lactose – 0·59, surcose – 0·68, galactose – 0·72, glucose – 0·77 and fructose – 0·81.

All the organisms utilized the mixture well. The results indicate that the dry weight of these fungi continued to increase till the end of the incubation period (as evident from the graph).

Of the five sugars in the mixture, glucose was utilized preferentially. *Isoachlya unispora, I. toruloides* and *Saprolegnia parasitica* consumed glucose entirely in 13,9 and 10 days respectively. *Achlya aplanes* was not able to finish this sugar even in 15 days. None of the fungi were able to consume the mixture entirely within the specified time.

TABLE 1

Showing the dry weight in mgm. of *Achlya aplanes, Isoachlya unispora, I. toruloides* and *Saprolegnia parasitica* in a mixture of five sugars.

Fungi	Days of Incubation		
	5	10	15
Achlya aplanes ...	0·7	5·4	12·5
Isoachlya unispora ...	4·2	16·4	18·4
I. toruloides ...	1·5	11·7	16·8
Saprolegnia parasitica ...	1·8	12·3	1 ·6

DISCUSSION

Lilly and Barnett (1951) have stated that a mixture of carbon sources may or may not result in an increased growth, depending upon the sugars involved and the fungus concerned. In the present studies the author observed that the fungi under investigation gave better yield on glucose alone than in the mixture of sugars where it was one of the constituents. Chromatographic studies revealed that glucose was utilized preferentially over other sugars from the medium, except in the case of *Achlya aplanes* where it persisted till the last day of incubation. It was interesting to note that though glucose was totally consumed from the medium on 9th, 10th and 14th day by *Isoachlya toruloides*, *Saprolegnia parasitica* and *I. unispora* but the dry weight attained by these organisms on the mixture was less than on glucose alone.

SUMMARY

1. The rate of growth and selective utilization of five sugars, viz., glucose, fructose, galactose, lactose and sucrose in a mixture by four fungi were studied.

2. None of the organisms grew better on the mixture of sugars than on glucose alone.

3. Glucose was preferentially utilized from the mixture as compared to other sugars.

4. None of the fungi could consume the mixture entirely within 15 days.

ACKNOWLEDGMENTS

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GRASSES OF JAIPUR

By

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INTRODUCTION

Jaipur, the capital city of Rajasthan, lies at the crossing of 76° longitude and 27° latitude in the North-Eastern part of the State. Unlike the North-Western region, which is characterised with intense arid conditions resulting from a series of undulating sand dunes, Jaipur and its neighbourhood, due to its semiarid climate develops a fair vegetation and in the mountain-surrounded valleys a deciduous forest develops during the monsoon season. The mountainous chains around the city are the extensions of the main Aravali ranges.

The city is flanked with Amber fort, Jaigarh fort, Nahargarh fort and Ganesh temple in the north, Laxman doongri, Ghat and Galta hill in the east, Rawalji-ka-bundh, Nallah gardens and a small township of Jhontwara on the west and Gandhi Nagar, Durgapura and Sanganer towns in the south (fig. 1).

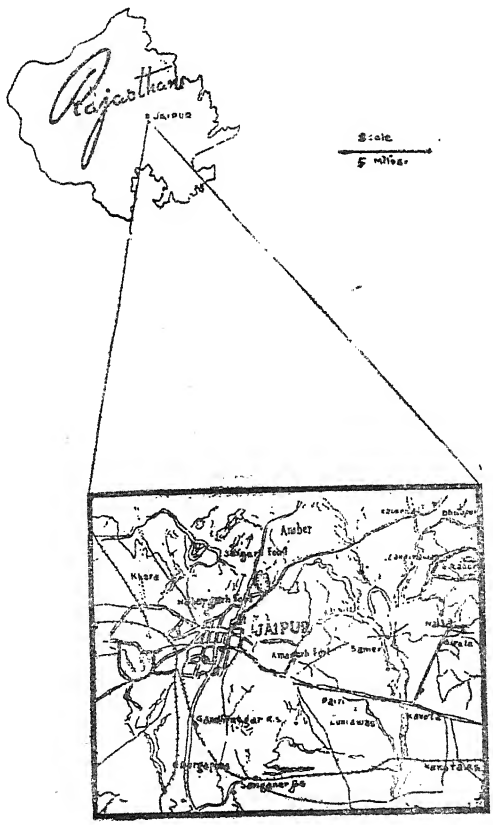

Fig. 1. Map of Jaipur

Meteorological data, given in table 1, indicate that May and June are the hottest dry months of the year when the mercury rises upto 103° and 105° F. and the relative humidity in the month of May even in the mornings is 37 percent. The humidity, of course, rises in the month of June, when monsoon starts. December, January and February are the cold months, when the lowest temperature runs upto about 46° F. with normal percentage of humidity.

TABLE 1

Monthly normals based on data for a large number of years (1951-58)

Month	Mean of		Relative Humidity %		Rainfall in inches.
	Daily Max. (F)	Daily Mini. (F)	0830 hrs. IST.	1730 hrs. IST.	
Jan.	73·2	46·8	59	29	0·44
Feb.	77·0	50·6	51	28	0·32
March	88·3	55·8	38	18	0·34
April	98·2	68·8	31	10	0·17
May	105·6	76·9	37	13	0·57
June	103·1	80·4	52	35	2·24
July	94·2	78·2	76	56	7·74
August	90·9	75·9	81	58	8·06
Sept.	93·1	72·8	73	44	3·22
Oct.	94·1	64·3	49	22	0·48
Nov.	85·4	53·7	49	25	0·14
Dec.	76·4	47·6	58	31	0·30
				Total ···	24·02

Monsoons start in the month of June and continue upto September, when a total of about more than 20 inches is received. In comparison to the arid regions of Rajasthan, Jaipur receives higher annual precipitation resulting in a semiarid climate. Winter rains are always scanty.

Soil analysis of Jaipur was done by Joshi (1957), when he conducted the studies on vegetation of some areas in Jaipur Division. Soil samples on Gandhinagar side (south) are sandy, which is mainly because of the sandy nature of the soil in this part of Jaipur. In other directions of the city the soil ranges from sandy to gravel and gritty types with varying degrees of humus content. On the whole the soil samples in Jaipur region are alkaline.

Various attempts have been made in early as well as in recent years to study the vegetation of Western and the Eatern part of Rajasthan by Pilani and Jodhpur school of workers, where post-graduate Botany classes exist. Many of these publications deal exaustively with the flora and ecology of the places but none is complete as far as Gramineae is concerned. An only publication describing the common grasses found round about Pilani, in the arid region of North-Eastern Rajasthan, was published by Ramachandran (1950).

With a view to study the detailed flora of Gramineae of Jaipur, where the climatic conditions differ from most of the parts in Rajasthan, collection tours were undertaken and the present paper is an outcome of these efforts extending over two to three years.

TAXONOMIC DATA

Subfamily : *Panicoideae*

1. *Apluda* Linn.

Apluda aristata Linn.

Annual. Appearance tufted. Grows in between the bushes and in old neglected places. Flowers in August-September.

2. *Manisuris* Linn.

Manisuris granularis Linn.

Annual. Not very common. Flowers in August-September.

3. *Imperata* Cyrill.

Imperata cylindrica (Linn) P. Beauv.

Perennial. Collected from the bank of the Nallah. Flowers in September-October.

4. *Saccharum* Linn.

Saccharum spontaneum Linn.

Perennial. Abundant on marginal dry lands or in the uncultivated kharif fields. Flowers in August-September.

Saccharum munja Roxb.

Perennial tall grass. Attains luxuriant growth near the nallah, where the moisture is available at higher level. Mostly used in thatching and also as soil binder. Flowers in October-November.

5. *Sorghum* Pers.

Sorghum halepense (Linn.) Pers.

Perennial. Very tall when grows with crop like Bajra. Also grows profusely in large tussocks wherever little moisture is available. Locally called Baru. Flowers in August-September.

6. *Dichanthium* Willemet

Dichanthium annulatum Stapf.

Densely tufted perennial grass. Very common as pasture grass. Also grows on roadsides on the farm. It is much relished by cattle. Flowers in August-September.

7. *Cymbopogon* Spreng.

Cymbopogon martini Stapf.

Perennial tall grass. Observed on Amber and Nahargarh side.

8 *Heteropogon* Pers.

Heteropogon contortus (L) Beauv.

Locally known as 'Surval'. Perennial. Grows in pure stand on farm wasteland and rocky places. Flowers during August-September.

9. *Digitaria* Hall.

Digitaria adscendens (H.B.K.) Henrard.

Annual. Quite common in cultivated fields. Also seen growing on the hills. Spikes two or more. Flowers in August-September. Good fodder grass.

10. *Alloteropsis* Presl. emend. Hitchcock

Alloteropsis cimicina Stapf.

A tufted grass. Inflorescence in spike like racemes. Collected at the base and up the hills. Flowers in rainy season.

11. *Erichloa* (H.B.K.)

Erichloa ramosa O. Kuntze.

Perennial. Densely tufted. Collected mostly from wet places in the garden. Also grows commonly at the base of the hills. Flowers in August-September.

12. *Brachiaria* Griseb.

Brachiaria ramosa Stapf.

An annual subgregarious grass. Flowers in August-October.

Brachiaria distachya Haines.

Annual. Slender creeping grass. Grows in marginal places, on paths and roadsides. Flowers from July to October.

13. *Paspalum* Linn.

Paspalum vaginatum Sw.

Perennial. Stem many noded and sheathed throughout. Collected from the sides of the ditch on the Govt. Farm. Flowers mostly in rainy season.

14. *Paspaladium* Stapf.

Paspaladium geminatum Stapf.

Perennial. Grows in marshy places. Collected from the nallah. Observed to flower in rainy season.

15. *Echinochloa* Beauv.

Echinochloa colona Link.

Annual. Slender long grass. Collections made from the cultivated fields. Relished greedily by all sorts of cattle. Flowers in August-September.

Echinochloa Crus-Galli P. Beauv.

Annual. Not very common. Grows in moist places. Flowers in August-September.

16. *Panicum* Linn.

Panicum psilopodium Trin.

Annual tufted grass. Grows on the boarders of cultivated fields. Flowers from August-September.

17. *Setaria* Beauv.

Setaria glauca Beauv.

Annual. Common in cultivated fields. Readily recognised by its cylindric inflorescence. Grazed by cattle. Flowers in rainy season.

Setaria verticillata Beauv.

Annual. Observed to grow in between other grasses. Panicle long and coarsely bristle and sticks readily to ones clothings. Flowers in August-September.

18. *Pennisetum* Pers.

Pennisetum ciliare Link.

Perennial. Locally known as Anjan. Exellent fodder grass. Prefers dry conditions. Grows on side paths in the fields. Flowers in August-September. When cultivated under irrigated conditions, gives high quality green fodder.

Pennisetum setosum Rich.

Annual. Collected from the down hills. Flowers in rainy season.

19. *Cenchrus* Linn.

Cenchrus biflorus Roxb.

Annual. Grows in sandy soils and uncultivated fields. Flowers in August-September. Very nutritive and excellent fodder grass.

Cenchrus catharticus Del.

Locally known as Bharont. Mostly inhabits sandy or arid soil. Flowers in rainy season and in some stray plants flowering was observed to continue even upto December.

Subfamily : *Pooideae*

20. *Aristida* Linn.

Aristida adscensionis Linn.

Annual. Very common, slender and densely tufted grass. Observed to grow on dry lands. Flowers in rainy season.

Aristida funiculata Trin. & Rupr.

Annual. Grows in association with other grasses. Not very common. Flowers during August-September.

Aristida mutabilis Trin. & Rupr.

Annual. Observed on dry patches. Flowers in August-September.

21. *Nazia* Adans.

Nazia racemosa Kuntze.

Annual. Collected from dry pastures. Not very common. Flowers during August-September.

22. *Perotis* Ait.

Perotis indica (Linn.) Q. Kuntze.

Annual. Commonly grows in sandy tracts, but develops nicely when it grows in association with other grasses. Flowers in monsoon season, easily recognised by its purple squirrel tail like inflorescence.

23. *Sporobolus* R. Br.

Sporobolus diander Beauv.

An annual, locally named as Chiri-ka-dana. Grows as a pasture grass on farm land in pure stands. Flowers in August-September.

24. *Eragrostis* Beauv.

Eragrostis ciliaris Link.

An annual tufted grass. Commonly grows on sandy soils. Develops nicely wherever good moisture is available. Collected from Durgapura garden. Flowers in August.

Eragrostis tenella P. Beauv.

Small slender annual grass. Observed on pasture grounds. Flowers in August-September.

Eragrostis minor Host.

Annual. Mostly found as weed in kharif crops and garden. Flowers in August.

Eragrostis tremula Hochst.

Annual. Grows particularly in dry habitats. Also observed as weed in kharif crops. Prefers sandy soil. Flowers in rainy season. Stray plants seen flowering even upto November and December.

25. *Desmostachya* Stapf.

Desmostachya bipinnata Stapf.

Perennial. Only species in the genus. Locally named as Dab and is considered as very sacred. Flowers with the first showers of monsoon rains. Perennation is by rhizomes which are four to five feet deep. Generally cattle do not eat it.

26. *Gracilea* Koen.

Gracilea royleana Hook.

Short statured annual grass. Collected from stony habitats in the hills. Flowers in rainy season.

27. *Cynodon* Rich.

Cynodon dactylon (L.) Pers.

Perennial. A very common lawn grass, which also grows as a weed and on the sides of the water channels. Locally called as Dub. Flowers throughout the year. When grows tall, used as pasture grass.

28. *Chloris* Swartz.

Chloris incompleta Roth.

Perennial. Flowers in rainy season. A very sporadic grass and collected from grass hedges, where it attains luxuriant growth.

Chloris tenella Kaen.

A slender annual grass. Flowers during August. Grows quite commonly on the sides of the fields. Used as fodder by the cattle.

Chloris montana Roxb.

Tufted perennial grass. Collected from the hilly habitats. Not liked by the cattle as fodder. Flowers during monsoons.

29. *Eleusine* Gaertn.

Eleusine indica Gaertn.

Erect annual grass. Flowers in rainy season during August. Grows by the roadsides and also in tufts on pasture lands. Mostly used as fodder.

Eleusine verticillata Roxb.

An annual grass of about a meter height. Flowers in August-September. Found to grow scattered on fields and in the gardens. Used as fodder.

30. *Dactyloctenium* Willd.

Dactyloctenium aegypticum Beauv.

A very common annual grass. Exhibits wide variability in growth depending on the availability of moisture. In dry sandy places, it shows stunted growth and assumes much prostrated habit. Flowers during August-September. Also grows as weed in kharif crops.

Dactyloctenium scindicum Boiss.

A typical annual desert grass. Completely spreading and covers large areas by stolons. Grows in sandy soils and slopes where the moisture supply is scanty. Flowers during August-September. When all vegetation disappears after rainy season, this grass is the last resort of the cattle

31. *Tripogon.*

Tripogon sps.

Small tufted grass. Collected from the flights of steps leading to Ganesh temple. Usually likes dry habitat.

DISCUSSION

Subfamily Panicoideae is represented in Jaipur by two tribes viz., Andropogoneae and Paniceae, each representing eight and eleven genera respectively. Among the genera represented by Paniceae, *Paspalum* and *Paspaladium* are hygrophilous, *Cenchrus* is xerophilous and the remaining genera have been mostly observed to grow in moist shady places. In contrast to Paniceae, genera of Andropogoneae exhibit much advanced ecological adaptations, since most of them inhabit dry situations. Except for *Imperata cylindrica* and *Saccharum munja*, which love to grow under higher moisture conditions, rest of the genera of Andropogoneae found in Jaipur are better able to endure semi-desert conditions.

Subfamily Pooideae is represented by five tribes, viz., Stipeae, Zoysieae, Sporoboleae, Eragrosteae and Chlorideae. Tribes Stipeae and Sporoboleae are represented by a single genus and the tribes Zoysieae and Eragrosteae by two genera each. Tribe Chlorideae alone includes as many as six genera, which equals the combined total of all the four remaining tribes of Pooideae. Genera of Pooideae are more prominent as desert grasses and important among them, like *Aristida*, *Nazia*, *Chloris*, *Desmostachya*, *Tripogon* and *Dactyloctenium scindicum* are very good indicators of extreme xerophytic conditions.

Details regarding the genera and species of grasses at Jaipur are given below.

Subfamily	Tribe	Genera		No. of species.
		Total No.	Names	
1	2	3	4	5
Panicoideae	Andropogoneae	8	Apluda	1
			Manisuris	1
			Imperata	1
			Saccharum	2
			Sorghum	1
			Dichanthium	1
			Cymbopogon	1
			Heteropogon	1
	Paniceae	11	Digitaria	1
			Alloteropsis	1
			Eriochloa	1
			Brachiaria	2
			Paspalum	1
			Paspalidium	1
			Echinochloa	2
			Panicum	1
			Setaria	2
			Pennisetum	2
			Cenchrus	2
Pooideae	Stipeae	1	Aristida	3
	Zoysieae	2	Nazia	1
			Perotis	1
	Sporoboleae	1	Sporobolus	1
	Eragrosteae	2	Eragrostis	4
			Desmostachya	1
	Chlorideae	6	Gracilea	1
			Cynodon	1
			Chloris	4
			Eleusine	2
			Dactyloctenium	2
			Tripogon	1
Total ...	7	31		47

SUMMARY

Ecological observations on the flora of Gramineae found in Jaipur have been recorded in the present paper. Out of the 31 genera collected from various parts of the city, 19 belong to the sub-family Panicoideae and 12 to Pooideae. All genera of Panicoideae belong to the tribes Andropogoneae and Paniceae. Pooideae is, however, represented by 5 tribes, two of which are monogenetic and two digenetic. Tribe Chlorideae alone constitutes of 6 genera. Most of the genera belonging to the tribes Andropogoneae and Paniceae love to grow under higher moisture conditions and the genera like *Paspalum* and *Paspaladium* exhibit the extremity of being hygrophilous. Genera of Pooideae are mostly extreme desert grasses.

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THE INVOLUTION OF THE BURSA OF FABRICIUS IN BIRDS*

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SUMMARY

1. The bursa of Fabricius in birds is a transitory organ which has no homologue in other classes of vertebrates.

2. The study of the involution of the bursa in the observed species supports the lympho-epithelial nature of the follicles as put forward by Jolly (1915).

3. The usual mode of involution of the follicles of the bursa is by pycnosis, gradual disappearance of the lymphocytes, retention of the epithelial reticulum and subsequent fibrosis.

4. Cystic degeneration of the follicles also occurs in which the entire medulla turns necrotic and the debris is discharged into the lumen of the bursa. This has not been reported by previous workers. Other types of cystic degeneration have also been noted.

5. All these types of degeneration are usually met with simultaneously in the follicles of the same bursa during involution.

6. As the bursa attains its maximum development in juvenile birds and begins to involute at the onset of sexual maturity, it is thought that there is an antagonism between the bursa and the gonad in birds.

7. Experimental work that has been done by some in order to prove the antagonism between gonad and bursa is discussed. The contradictory results of castration and bursectomy, which have not yielded any indication of the function of the bursa, are also discussed.

INTRODUCTION

The bursa of Fabricius is an organ peculiar to birds. It is a sac-like outgrowth from the dorsal wall of the proctodaeum of the cloaca and reaches its maximum development in juvenile birds. It begins to involute at the onset of sexual maturity and completely disappears in the adult birds.

*Part of a thesis approved for the Degree of Doctor of Philosophy of the Banaras Hindu University in 1959.

Although much is known about the structure and development of the bursa of Fabricius (Jolly, 1915; Boyden, 1922; Mathis, 1938; Ackerman and Knouff, 1959 and Dominic, 1959-1960 *a* and *b*), account of the involution of this organ is somewhat inadequate. Jolly (1911, 1913 and 1915) studied in some detail the changes taking place in the bursa during its involution and pointed out its relation with the advent of sexual maturity. Masi (1922) found that the bursa begins to involute at the approach of sexual maturity in the domestic fowl. The inverse relationship existing between the bursa and the gonads has also been pointed out by Gower (1939), Hoschbaum (1942) and Linduska (1943). Selye (1943), Kirkpatrick and Andrews (1944) and Rao, Aspinall and Buchanan (1958) have brought about the premature involution of the bursa by the injection of steroids. In this paper an attempt is made to study in detail the histological changes that take place in the bursa of Fabricius during its involution.

MATERIAL AND METHODS

Fifty-seven species of Indian birds belonging to thirteen Orders and twenty-nine Families were used in this study. The names of the species studied are given in another paper (Dominic, 1959-60*b*) which is published elsewhere. Birds were killed by decapitation, and the bursa immediately removed and fixed in appropriate fluids like Allen's B-15, Bouin's, Zenker, Helly's Zenker and Susa. The material was embedded in paraffin, sectioned at 5 or 7½ μ and stained mostly in Heidenhain's iron haematoxylin and eosin or Mallory's triple stain. Delafield's haematoxylin, Heidenhain's Azan stain and Mann's methyl blue-eosin were also tried. The condition of the gonads was also noted.

OBSERVATIONS

Gross Morphology of the Bursa of Fabricius :

The bursa is enclosed in a sheath of circular muscle fibres. Internally the lumen of the bursa is lined by an epithelium which is continuous with that of the cloaca. In the space between the lining epithelium and the muscular coat are found the lymphoid follicles of the bursa. Each follicle has a lightly staining area called the medulla and a somewhat darkly staining area called the cortex. The medulla is composed of both lymphocytes and endodermal epithelial cells, whereas the cortex contains only lymphocytes. The cortex and the medulla are separated by a layer of connective tissue. The follicles are surrounded by connective tissue trabeculae in which are present blood capillaries. All the follicles of the bursa retain a link with the lining epithelium by means of an epithelial invagination called the 'collar'.

The Involution of the Bursa of Fabricius :

The mechanism of involution is practically the same in all the species studied. It consists in the degeneration and disappearance of the lymphocytes from the follicles and the consequent fibrosis and gradual disappearance of the bursa. Cystic degeneration of the follicles is also not uncommon.

The first sign of the involution of the bursa is given by the lymphocytes of the medulla of the follicles which become pycnotic and begin to disappear (Pl. I,

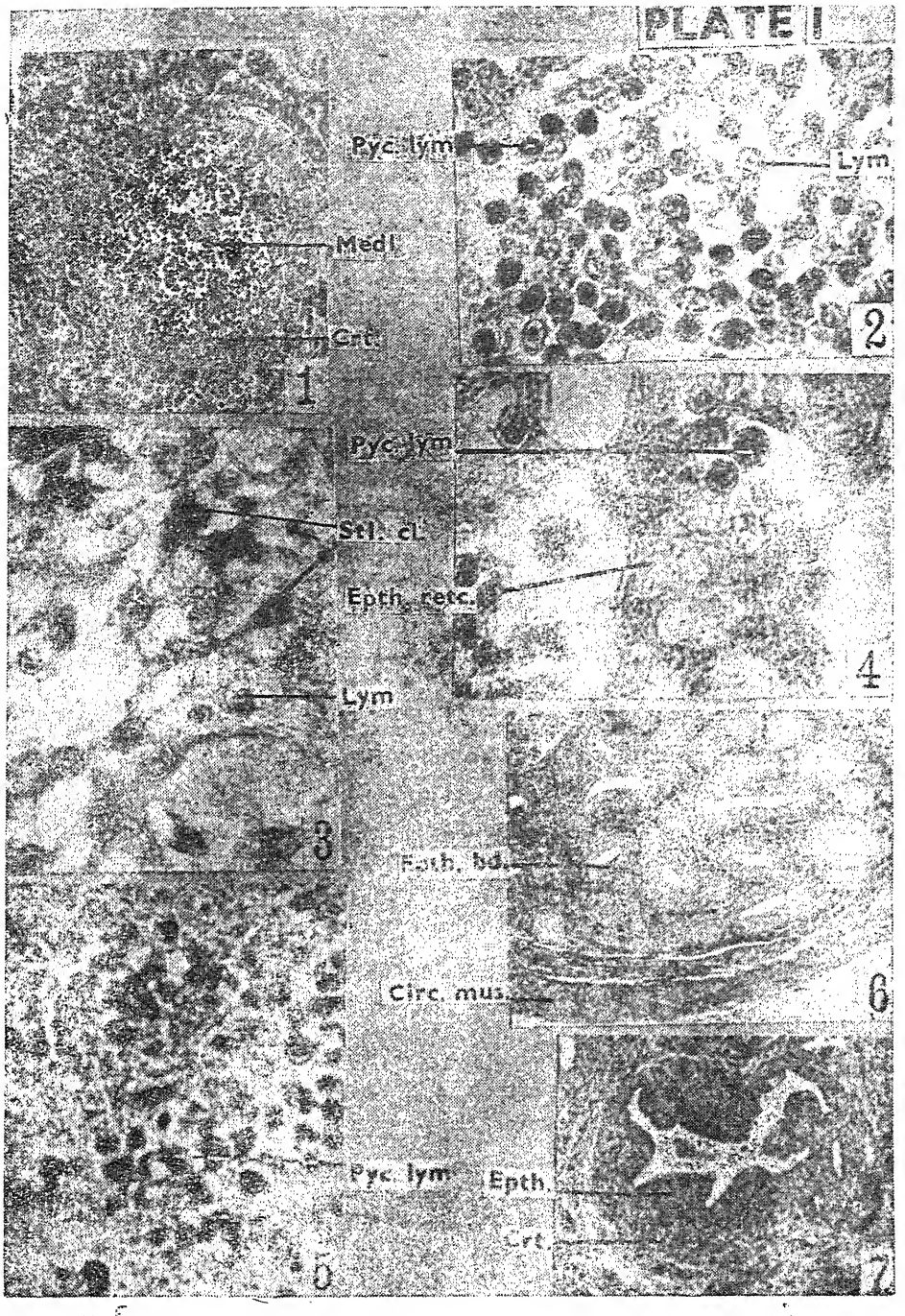


PLATE I
Pyc. lym
Lym
Medl
Cnt.
1
2
Pyc. lym
Stl. cL
Epth. retc.
Lym
4
3
Epth. bd.
Circ. mus.
6
Pyc. lym
Epth.
Cnt.
5
7

Figs. 1 and 2). As a result of such degeneration, the medulla becomes less compact and spaces appear in it (Pl. I, Figs. 3 and 4). As involution proceeds, more and more lymphocytes undergo pycnosis and become eliminated. Such degeneration, however, does not affect the endodermal epithelial cells; instead they become more prominently visible. The epithelial cells very in size. They usually retain a large quantity of eosinophilic cytoplasm which is produced into 'rays' or strands (stellate cells) often anastomosing with those of the neighbouring cells to form a kind of network (Pl. I, Figs. 3 and 4). This epithelial reticulum is continuous with the epithelium of the collar and also with the epithelial cells lying inner to the layer separating the cortex from the medulla. In the meshes of the network are contained the lymphocytes (Pl. I, Figs. 3 and 4). Sometimes multinucleate knots of epithelial cells are found in the epithelial reticulum. As regression of the follicle proceeds, the layer separating the cortex from the medulla becomes better defined.

Along with these changes in the medulla, the lymphocytes in the cortex also turn pycnotic (Pl. I, Fig. 5), and disappear and, consequently the cortex becomes very thin.

As involution proceeds further, the follicle shrinks in size and the medulla assumes a reticular form. Most of the lymphocytes in the medulla become pycnotic and disappear leaving behind a network in which the remaining lymphocytes are lodged. The stellate epithelial cells are very prominently visible. Gradually these cells shrink in size, their arms or 'rays' are shortened and the medulla as a whole becomes more compact resulting in the formation of an epithelial 'bud' (Pl. I, Fig. 6) which places it in direct continuity with the epithelium lining the lumen of the bursa. During these changes in the medullary region of the follicle, the cortex also degenerates by the pycnosis of its lymphocytes and is ultimately represented by a thin layer of lymphoidal tissue encircling the epithelial bud (Pl. I, Fig. 7). Later on this also disappears. As involution progresses still further, the epithelial bud shrinks furthermore and finally disappears altogether.

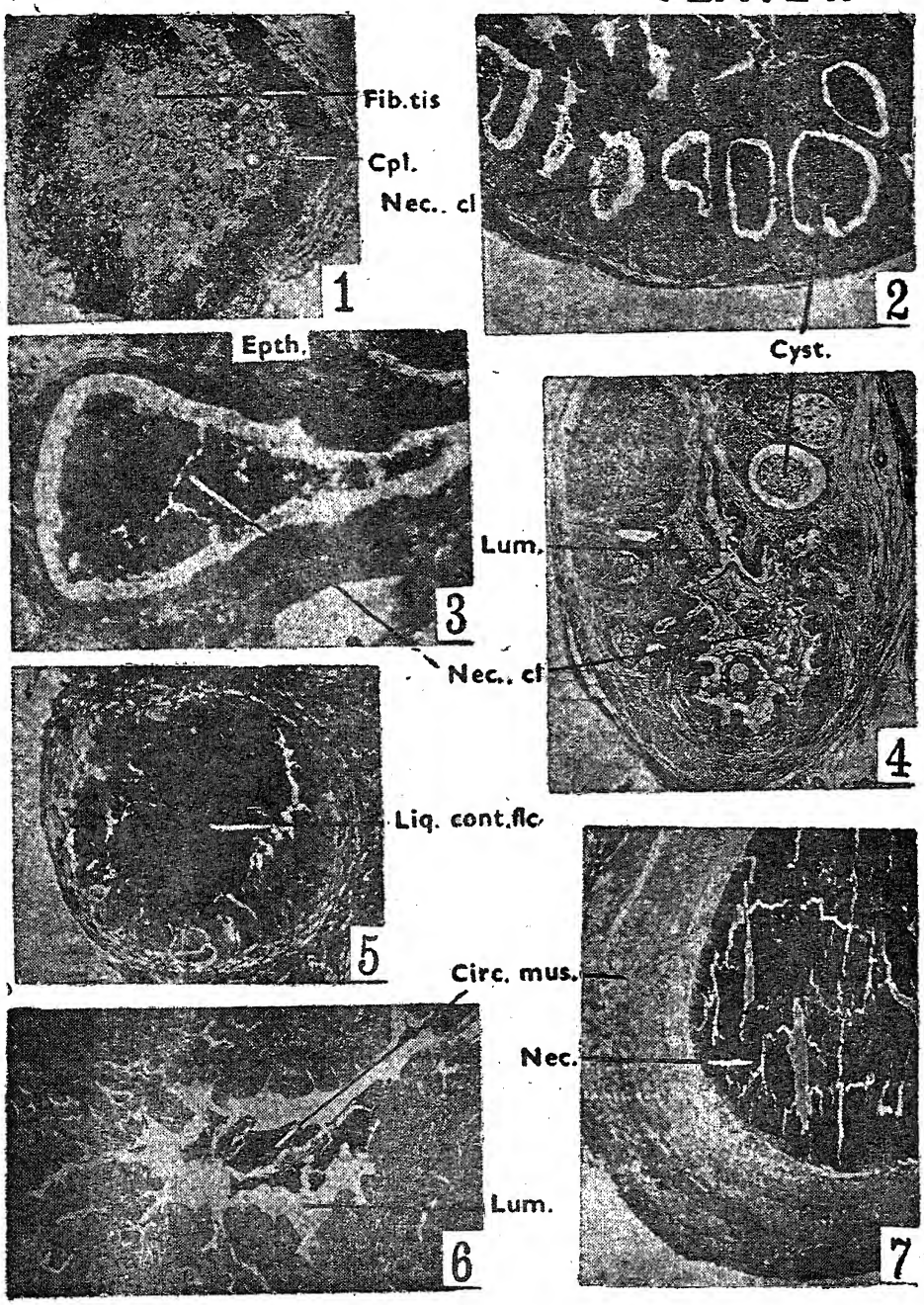
By reason of the involution of the follicles, the bursa gradually shrinks in size, the lymphoidal tissue disappears and fibrosis takes place (Pl. II, Fig. 1). The fibrosis begins at the apex of the bursa and advances towards its base. As a consequence of this fibrosis, the lumen of the bursa is lost. A few blood capillaries may be present in the fibrous tissue. The bursa finally assumes the form of a very small conical structure on the dorsal wall of the cloaca. Often this also disappears, and no trace of it is left behind on the cloaca to indicate its presence in the adult bird.

Occasionally during involution, hyaline degeneration of the buds and the bursa as a whole takes place. When this kind of degeneration proceeds far enough, the bursa becomes a hyaline mass containing a small lumen which becomes obliterated later. Small darkly staining nuclei may be left behind in the hyaline mass.

Besides the type of involution described above, follicles may undergo cystic degeneration also. This type of involution may be found alongside the previous one in the follicles of the same bursa, or, in an extreme case all the follicles of the bursa may undergo cystic degeneration simultaneously. Cystic degeneration of the follicles may be one of three kinds.

(i) The cellular contents of the medulla of a follicle (including the lymphocytes and the epithelial cells) turn necrotic, shrinks and form a kind of cyst leaving a

PLATE II


Fib.tis
Cpl.
Nec.. cl
1
2
Cyst.
Epth.
3
Lum.
Nec.. cf
4
Liq. cont.flc
5
Circ. mus.
Nec.
Lum.
6
7

thin and flattened epithelium to line the wall of the cyst (Pl. II, Fig. 2). The epithelium of the 'collar' also degenerates and thus the cavity of the cyst becomes continuous with the lumen of the bursa (Pl. II, Fig. 3). The bulk of the necrotic cells escapes into the lumen of the bursa whence they escape into the cloaca and become eliminated. A large quantity of cellular debris resulting from the degenerating follicles is often found in the lumen of the bursa (Pl. II, Fig. 4) as a result of the cystic degeneration. Simultaneously, the lymphocytes in the cortex also degenerate and disappear.

(*ii*) The contents of a follicle liquefy into a substance which has a great affinity for iron haematoxylin (Pl. II, Fig. 5). The liquefaction begins at the centre of the follicle and proceeds outwards to the periphery. No cellular elements are recognisable in the liquefied mass which ultimately makes its way into the lumen of the bursa (Pl. II, Fig. 6).

(*iii*) Occasionally, the whole of the interior of the bursa, except the muscular coat, undergoes necrosis resulting in the production of a mass of matter which stains intensely with iron haematoxylin (Pl. II, Fig. 7). This mass is contained in the space bounded by the muscular coat of the bursa. As involution proceeds, the bursa shrinks in size and the necrotic mass escapes into the cloaca. As a result of this the bursa is reduced to a thin tubular structure containing a spacious empty lumen. This kind of involution is, however, rare as it was observed only in the spotted owlet (*Athena brahma*), the seven sisters (*Turdoides terricolor*) and the shikra (*Astur badius*).

These various kinds of involution of the follicles usually occur side by side in the same bursa of Fabricius.

DISCUSSION

The bursa of Fabricius has long been recognized as a transitory organ which has no homologue in other classes of vertebrates. Occasionally it tends to persist in the adult birds also. Only in the Ratitae, according to Forbes (1877), the bursa persists throughout life, and it opens into the proctodaeum by a broad aperture.

The essentials of the involution of the follicles of the bursa of Fabricius agree in most respects with the findings of Jolly (1915) and support the belief in the lympho-epithelial nature of the follicles. During the development of the follicles (Jolly, 1915; Boyden, 1922 and Dominic, 1959-1960 a) the epithelial buds are invaded by mesenchyme cells to form the medulla, but the cortex is formed from the mesenchymal cells alone. On account of the dual origin of the follicles, the bursa is viewed as a lympho-epithelial organ (Jolly, 1915). During involution the lymphocytes are discarded (Pl. I, Figs. 1 and 2) and the epithelial reticulum shrinks to reconstitute itself into a compact epithelial bud (Pl. I, Fig. 6). Thus we see that during involution, the order of events is reversed.

The modes of involution of the bursa of Fabricius, as observed in the present investigation agree in many respects with the processes described by Jolly (1915). The usual mode of involution of the follicles is by pycnosis, gradual disappearance of the lymphocytes (Pl. I, Figs. 1 and 2), retention of the epithelial reticulum (Pl. I, Figs. 3 and 4) and the subsequent fibrosis (Pl. II, Fig. 1). In addition to this type, cystic degeneration of the follicles also occurs (Pl. II, Figs. 2—7). The type of cystic degeneration (Pl. II, Figs. 2, 3 and 4) noted by the present author in which the entire medulla turns necrotic and the resulting debris is discharged into the

lumen of the bursa has not been reported by any of the previous workers. Jolly (1915) described the formation of cysts by the degeneration and liquefaction of the follicles in the bursa. This type of cystic degeneration was also noticed by the present author (Pl. II, Figs. 5 and 6). It is interesting to note that, in the same bursa, one may come across follicles undergoing different types of degeneration. The type of degeneration (Pl. II, Fig. 7) found in *Athena brahma*, *Astur badius* and in *Turdoides terricolor* in which the entire content of the bursa, except the muscular coat, turns necrotic was only described in an individual fowl by Jolly (1915) who suspected it to be due to the action of some parasites.

The causal relationship between sexual maturity and the involution of the bursa of Fabricius has been pointed out by many workers. In the species studied by the present author, the bursa was seen to be completely involuted in the adults. This is in agreement with the findings of Jolly (1915), Riddle (1928), Gower (1939), Hoschbaum (1942), Linduska (1943), Kirkpatrick (1944) and Hohn (1957). Riddle found that the growth and involution of the bursa coincide with certain events in the life of the birds, because "it grows while the thymus grows; while the body is rapidly growing; and while growth in gonads in obviously repressed. It begins involution when the thymus begins involution; when adult weight is nearly attained; and when the gonads begin a more rapid development. The involution of the bursa is usually completed—that is, it usually disappears—coincident with sexual muturity. It tends to persist longest in birds which mature latest. These associations seem to suggest an endocrine function for this particular organ." This is in agreement with the view of Jolly (1915) that the bursa of Fabricius produces certain substances which have a causal relationship with sexual maturity.

There is experimental evidence also to prove the inverse relationship between the bursa and the gonads. The injection of steroids bring about the premature involution of the bursa in sexually immature birds (Selye, 1943; Kirkpatrick and Andrews, 1944; Rao, Aspinall and Buchanan, 1958).

However, castration and bursectomy give contradictory results. Jolly and Pezard (1928) found that the castration of the male domestic fowl had resulted in the delayed involution of the bursa. This is contrary to the findings of Kirkpatrick (1944), who reported that castration had no apparent effect upon the rate of involution of the bursa of the ring-necked pheasant. Riddle and Tange (1926) and Taibel (1938) found that bursectomy had no apparent effect on the timing of sexual maturity in birds. Thymo-bursectomy by Riddle and Krizenecky (1931) also failed to produce any perceptible effect. Hence they conclude that the thymus and the bursa of Fabricius have functions similar to other lymphoidal tissues and believe that tissues with a sort of 'thymic function' are widely distributed in the body of vertebrates.

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EXPLANATION OF PLATES

PLATE I

Fig. 1: Photomicrograph of a follicle of the bursa of *Passer domesticus* showing the pycnotic lymphocytes in the medulla × 220.

Fig. 2: Photomicrograph of the medulla of *Streptopelia tranquebarica* showing the pycnotic and healthy lymphocytes × 1060.

Fig. 3: Photomicrograph of the medulla of *Corvus macrorhynchus* showing lymphocytes and stellate epithelial cells forming a reticulum × 900.

Fig. 4: Photomicrograph of the medulla of the follicle of *Columba livia* showing the lymphocytes in the meshes of the epithelial reticulum × 1080.

Fig. 5: Photomicrograph of a part of the cortex of the follicle of *Xantholaema haemacephala* showing the pycnotic lymphocytes × 1120.

Fig. 6: Photomicrograph of a part of the transverse section of the bursa in *Acridotheres ginginianus* showing the epithelial buds formed after the disappearance of the lymphocytes during involution × 160.

Fig. 7: Photomicrograph of the involuted follicle of *Dicrurus macrocercus* showing the epithlial lining and the thin cortex × 160.

PLATE II

Fig. 1: Photomicrograph of the transverse section of the involuted bursa of *Columba livia* showing the fibrous tissue and the capillaries × 30.

Fig. 2: Photomicrograph of a portion of the transverse section of the bursa of *Dicrurus macrocercus* showing the cystic degeneration of follicles × 60.

Fig. 3: Photomicrograph of the cystic degeneration of a follicle of *Dicrurus macrocercus* showing the discharge of the follicular debris into the lumen of the bursa × 240.

Fig. 4: Photomicrograph of the transverse section of the involuting bursa of *Dicrurus macrocercus* showing the lumen containing the discharged debris of the follicles × 45.

Fig. 5 Photomicrograph of the involuting follicle of *Streptopelia senegalensis* showing the follicle in a state of liquefaction × 130.

Fig. 6: Photomicrograph of the involuted bursa of *Dicrurus macrocercus* showing the lumen with the discharged debris of involuted follicles × 50.

Fig. 7: Photomicrograph of the transverse section of the bursa of *Athene brahma* during involution showing the muscular coat and the lumen containing the necrotic mass derived from the degeneration of the entire bursa × 30.

ABBREVIATIONS USED

Cpl.—Capillary; Circ. mus.—Circular muscle; Crt.—Cortex; Cyst.—Cyst; Epth.—Epithelium; Epth. bd.—Epithelial bud; Epth. retc.—Epithelial reticlulum; Fib. tis.—Fibrous tissue; Liq. cont. flc.—Liquefied content of follicle; Lum.—Lumen; Lym.—Lymphocytes; Medl.—Medulla; Nec.—Necrotic content of bursa; Nec. cl.—Necrotic cells; Pyc. lym.—Pycnotic lymphocytes; Stl. cl.—Stellate epithelial cells.

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A SYSTEMATIC ACCOUNT OF THE FRESH-WATER DIATOMS OF UTTAR-PRADESH—II

(Diatom-flora of the Banaras Hindu University)

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INTRODUCTION

Studies on the systematics of Diatom-flora have been done in some detail from various parts of India. Surprisingly enough such studies from Uttar Pradesh are, however, altogether lacking. Therefore, investigations have been undertaken by the author from Uttar Pradesh. In a previous paper which is under publication, 33 forms of Diatoms have been described from a permanent pond of district Deoria, United Provinces.

The present paper deals with the systematics of 58 Diatoms found in some temporary ponds existing in the campus of the Banaras Hindu University.

MATERIALS & METHOD

Banaras Hindu University lies at 25·18′ N. lat. and 83·1′ E. long. in the eastern part of the upper gangetic plain which constitutes a distinctive floristic sub-division of Uttar Pradesh. It is about 252 feet above the mean-sea-level, and has three well marked seasons namely the rainy, the winter and the summer with an average annual rainfall of about 40″. Some important climatological data for the period of this investigation are given in the table no. 1.

TABLE No. 1

Climatological data for 1959-60

Month	Mean daily Max. Temp. F.	Abs. Max. Temp. F.	Mean daily Min. Temp. F.	Abs. Min. Temp. F.	Rainfall in Inches	No. of Rainy days	Mean relative humidity at 8 a. m.
1959							
July ...	92·9	101·4	79·4	73·8	10·67	15	83
Aug. ...	90·5	95·9	78·8	76·1	10·29	17	87
Sept. ...	91·7	99·9	77·6	73·0	7·18	12	84
Oct. ...	89·9	96·0	72·2	64·6	5·17	7	85
Nov. ...	80·2	93·1	56·9	49·5	Nil	Nil	72
Dec. ...	79·6	86·2	48·9	42·6	Nil	Nil	72
1960							
Jan. ...	74·8	81·5	49·1	40·7	0·38	5	76
Feb. ...	87·7	95·5	53·7	43·7	Nil	Nil	60
March ...	86·2	100·1	58·2	58·2	0·25	3	55·5
April ...	105·05	109·2	25·0	25 0	0·50	2	56·1
May ...	109·4	114·5	79·35	66·0	0·04	1	63
June ...	107·17	116·5	82·4	60·0	1·6	3	66

The ponds studied were mostly shallow and temporary, holding rain waters from July upto May, and drying completely during the month of June. During the winter months the water in all the ponds remains enough to support a crop of *Trapa natans* and also the growth of *Eleocharis plantaginea* and *Echinops echinates* along the gradually exposed margins.

The conditions essential for the growth of diatom appear to be moisture and light. Since it has been observed that two identical diatoms were comparatively more abundant in parts well exposed to the radiation of the sun, than in shady places. While both being absent from below the euphotic regions of the water contained in the ponds. The density of the diatoms is also found to be maximum in the inner parts of the ponds. But it decreasees towards margins with shallow waters where bacteria are found in excess. In each of the three zones namely (1) margin (2) centre and (3) intermediate regions of the ponds, and the density is higher at the bottom.

In all 20 genera representing 58 species have been described, of these 2 are new species, 1 new variety and 1 new form.

The classification by Hustedt (1930) has been followed.

A SYSTEMATIC ENUMERATION OF THE DIATOMS

BACILLARIOPHYTA

A. Order	...	...	CENTRALES
Sub-order	...	...	DISCINEAE
Family	...	...	Coscinodiscaceae
Sub-family	...	...	Melosiroideae
Genus	...	...	MELOSIRA Agardh, 1824.

1. *Melosira granulata* (Ehr.) Ralfs.

(Fig. 1.)

Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 87, fig. 44; Venkataraman, G., Systematic Account of some South Indian Diatoms, Pro. Ind. Acad. Sci., 1939, Vol. X, p. 296, fig. 1.

Melosira polymorpha sub-species *granulata* (Ralfs.) Bethgae; Bethage Kolkwitz Planzenforschung, Heft 3, 1925, p. 30, Tafel 1, fig. 1.

Frustules cylindrical, robust and stiff in detached filaments. Mantle portions cylindrial, discs flat. Mantle line straight, parallel. Mantle surface punctate, punctae coarse in more or less sprial rows. The outer shell always coarsly punctate, their puncta rows being parallel. The same cells have spines projecting outside as well as inside the cells.

Dimensions :—

Diameter	...	... 11·5 - 14 μ
Height of the half cell		... 11·5 – 13·5 μ
Rows of the punctae in the upper cell		... 9 – 10 in 10 μ
No. of the punctae in the upper cell		... 9 – 10 in 10 μ
Rows of the punctae in the lower cell		... 11 – 12 in 10 μ
No. of the punctae in the lower cell		... 11 – 12 in 10 μ

Habitat :—Fresh-water pond behind the B. H. U. Press. This specimen agrees with the type well as described in the literatures.

2. *Melosira granulata* (Ehr.) Ralfs. var. *angustissima* Müll.

(Fig. 2)

Diameter 4 - 5 μ mostly 4·5 μ, height of the half cell 11 – 12 μ, rows of punctae in the upper cell 9 – 10 in 10 μ, No. of punctae in the upper cell 9 - 10 in 10 μ, rows of punctae in the lower cell 10 - 12 in 10 μ, No. of punctae in the lower cell 10 – 12 in 10 μ.

Sub-family	...	... Coscinodiscoideae
Genus	...	... CYCLOTELLA Kützing, 1834.

3. *Cyclotella Meneghiniana* Kütz.

(Figs. 3,4)

Diameter 9·5 – 11·5 μ and striations 8 - 10 in 10 μ

4. *Cyclotella operculata* (Ag.) Kütz.

(Fig. 5)

Diameter 6·5 - 11 μ and striations 13 – 16 in 12 μ.

B.	Order	...	... PENNALES
	Sub-order	...	... ARAPHIDINEAE
	Family	...	... Fragilariaceae
	Sub-family	...	... Fragilarioideae
	Genus	...	... FRAGILARIA Lyngbye, 1819.

5. *Fragilaria crotonensis* Kitton

(Fig. 6)

Length 100-133 μ, breadth 3·5-4·5 μ, and striations 15-17 in 10 μ.

Genus ... ... SYNEDRA Ehrenberg, 1830

6. *Synedra berolinensis* Lemmermann

(Fig. 7)

Hüstedt, Fr., Rabenhorst's Kryptogamen-Flora, Teil 2, p. 184, fig. 686; Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 164, fig. 200.

Frustules in the girdle-view linear, like a clusters in the colonial form. Valves linear, very slight swelling in the middle, ends stumpy like *Fragilaria pinnata*. Striae small and clear. Pseudoraphe narrow, linear but distinct, central area absent.

Dimensions :—

Length	...	... 46 – 50·5 μ
Breadth	...	... 3 μ
Striations	...	... 12 – 14 in 10 μ

Habitat :—Fresh-water pond behind the B. H. U. Press. This form agrees in all respects with the type but the present form is a longer one than the type which is only 5 – 40 μ long and 3 μ broad.

7. *Synedra tenera* W. Smith

(Fig. 8)

Length 87 – 100 μ, breadth 2 – 3 μ and striations 15 – 20 in 10 μ.

8. *Synedra ulna* (Nitzsch) Ehr.

(Figs. 9,10)

Length 90-100 μ, breadth 4-4·5 μ and striations 10-12 in 10 μ.

Sub-order	...	... RAPHIDIOIDINEAE
Family	...	... Eunotiaceae
Sub-family	...	... Eunotioideae
Genus	...	... EUNOTIA Ehrenberg, 1837.

9. *Eunotia alpina* (Naeg.) Hüst.

(Figs. 11,12)

Length 40-50 μ, breadth 1·5-2·5 μ and striations 16-17 in 10 μ.

10. *Eunotia proerupta* Ehr. var *inflata* Grun.

(Fig. 13)

Length 25-30·5 μ, breadth 6·5-7·5 μ and striations 10-12 in 10 μ.

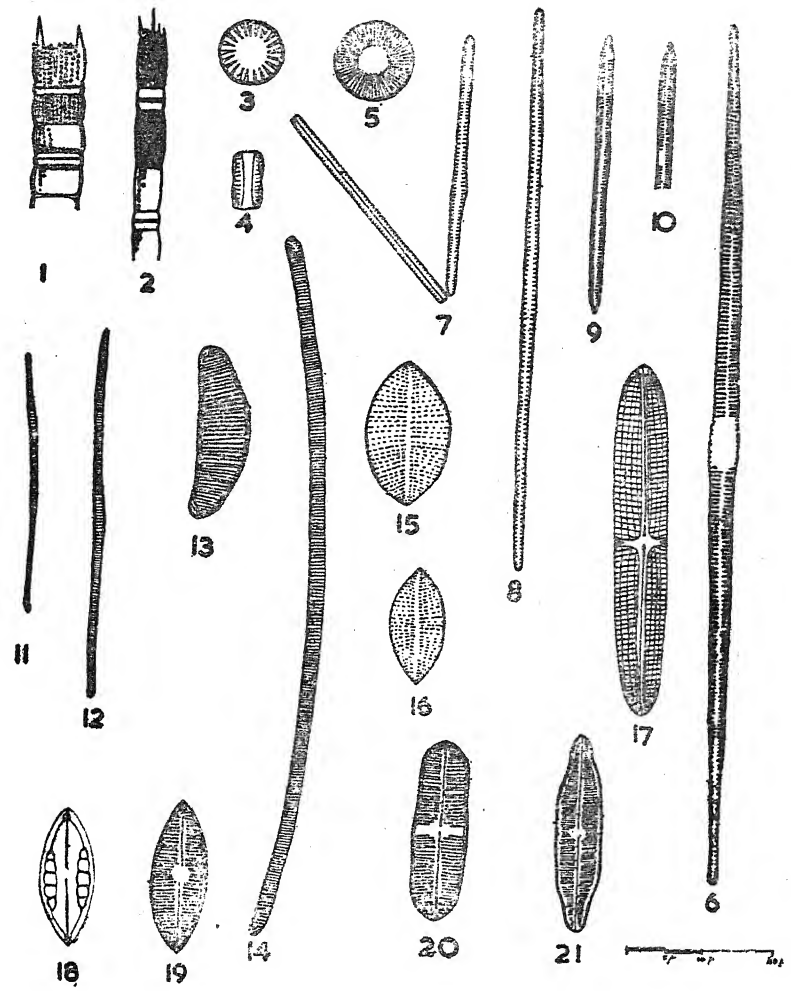


PLATE No. I

Figs. 1-21. Fig. 1. *Melosira granulata* (Ehr.) Ralfs. × 1500. Fig. 2. *Melosira granulata* (Ehr.) Ralfs. var. *angustissima* Müll. × 1500. Figs. 3, 4. *Cyclotella Meneghiniana* Kütz. × 1500. Fig. 5. *Cyclotella operculata* (Ag.) Kütz. × 1500. Fig. 6. *Fragilaria crotonensis* Kitton × 1500. Fig. 7. *Synedra berolinensis* Lemmer. × 1500. Fig. 8. *Synedra tenera* W. Smith × 1500. Figs. 9, 10. *Synedra ulna* (Nitz.) Ehr. × 1500. Figs. 11, 12. *Eunotia alpina* (Näeg.) Hüst. × 1500. Fig. 13. *Eunotia proerupta* Ehr. var. *inflata* Grun. × 1500. Fig. 14. *Eunotia pseudolunaris* Venk. × 1500. Figs. 15, 16. *Cocconeis plancentula* Ehr. var. *euglypta* (Ehr.) Cleve × 1500. Fig. 17. *Achnanthes septata* Cleve, A., × 1500. Figs. 18, 19. *Mastogloia exigua* Lewis for. *brevirostris* Venkataraman × 1500. Fig. 20. *Coloneis bacillum* (Grun.) Mereschkowsky × 1500. Fig. 21. *Neidium affine* (Ehr.) Cleve var. *longiceps* (Greg.) Cleve × 1500.

11. *Eunotia pseudolunaris* Venkat.

(Fig. 14)

Length 102-108·5 μ, breadth 3-3·5 μ and striations 15-16 in 10 μ.

Sub-order	...	... MONORAPHIDINEAE
Family	...	... Achnanthaceae
Sub-family	...	... Cocconeoideae
Genus	...	... COCCONEIS Ehrenberg, 1838.

12. *Cocconeis placentula* Ehr. var. *euglypta* (Ehr.) Cleve

(Figs. 15,16)

Length 13·5-21·5 μ, breadth 7·5-13·5 μ and striations 16-18 in 10 μ.

Sub-family	...	... Achnanthoideae
Genus	...	... ACHNANTHES Bory, 1822.

13. *Achnanthes septata* A. Cleve

(Fig. 17)

Length 51·5-55 μ, breadth 8-10 μ and striations (long.) 9-10 in 10 μ and trans. striations 8-9 in 10 μ.

Sub-order	...	... BIRAPHIDINEAE
Family	...	... Naviculaceae
Sub-family	...	... Naviculoideae
Genus	...	... MASTOGLOIA Thwaites, 1856.

14. *Mastogloia exigua* Lewis for. *brevirostris* Vekat.

(Figs. 18,19)

Length 25·5-32 μ, breadth 8·12 μ, striations 17-19 in 10 μ, length of the longer loculi 4 μ and length of the smaller loculi 2 μ.

Genus	...	... CALONEIS Cleve, 1894.

15. *Caloneis amphisbaena* (Bory) Cleve, for. *indicum* for. nov.

(Fig. 22)

Valvulae elliptico-lanceolatae, apicibus bene productis, contrictis, capitatis; superficies valvulae rotundata usque ad polos. Area axialis linearis, centralis vero ampla, elliptica. Raphe robusta et recta, poris centralibus eminentibus. Striae distinctae, radiales ad medium, convergentes ad apices. Zona marginalis unica adest. Long 56·5-59·5 μ; latit 20-22 μ; striae 18-20 in 10 μ.

Valves elliptical-lanceolate with well produced, constricted capitate ends, surface of the valve rounded upto the poles. Axial area linear, central area big, elliptic. Raphe strong and straight with prominent central pores. Striae clear, radial in the middle and convergent towards the ends. A single marginal band is present.

Dimensions :—

Length	...	... 56·5 – 59·5 μ
Breadth	...	... 20 – 22 μ
Striations	...	... 18-20 in 10 μ

Habitat :—Fresh-water pond near Engineering College B. H. U.

The present form agrees with *C. amphisbaena* (Bory) Cleve (Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 230, fig. 346) but distinguished by (i) the ends are fairly capitate, (ii) central area elliptical and (iii) raphe straight but not complex. Hence, the present species is regarded as a new form.

16. *Caloneis bacillum* (Grun.) Mereschkowsky

(Fig. 20)

Length 22·5-26·5 μ, breadth 5·5-7·5 μ and striations 22-25 in 10 μ.

Genus ... ... NEIDIUM Pfitzer

17. *Neidium affine* (Ehr.) Cleve var. *longiceps* (Gregory) Cleve

(Fig. 21)

Length 30·5-32·5 μ breadth 7·5-9 μ and striations 18-21 in 10 μ.

Genus ... ... STAURONEIS Ehrenberg, 1843.

18. *Stauroneis anceps* Ehr. for. *gracilis* (Ehr.) Cleve

(Fig. 23)

Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 256, fig. 406 ; Gonzalves, E. A., and Gandhi, H. P., Diatoms of Bombay and Salsette-II, J.I.B.S., 1953, 32, p. 257, fig. 94.

Valves lanceolate, gradually tapering from middle towards the poles, which are capitate. Raphe thin and straight. Axial area narrow, central area linear, stauroid. Striae very indistinctly punctate, strongly radial.

Dimensions :—

Length	...	... 60·5-76·5 μ
Breadth	...	... 10-16·5 μ
Striations	...	... 18-20 in 10 μ

Habitat :—Fresh-water pond near Agriculture College Farm B. H. U. This form resembles with the type in all respects, but is a smaller form.

19. *Stauroneis anceps* Ehr, var. *hyline* Brun. & Peragallo

(Fig. 24)

Length 60-82·5 μ, breadth 10-13·5 μ and striations 26-28 in 10 μ.

20. *Stauroneis phoenicenteron* Ehr.

(Fig. 25)

Length 69-85 μ, breadth 15·5-18 μ and striae 18-20 in 10 μ.

Genus	...	...	NAVICULA Bory, 1822.
Section	...	...	Naviculae orthostichae Cleve

21. *Navicula cuspidata* Kütz. var. *conspicua* Venkat.

(Figs. 26,27)

Length 124-130 μ breadth 28-30 μ, trans. striations 15-19 in 10 μ and long. striations 9-10 in 10 μ.

22. *Navicula cuspidata* Kütz. for *indica* Krishnamurthy

(Fig. 28)

Length 70-100 μ, breadth 15·5-26-25 μ and striations 13-15 in 10 μ.

23. *Navicula spicula* (Dickie) Cleve var. *minima* var, nov.

(Fig. 30)

Valvulae angustae linear-lanceolatae, apicibus obtusis. Area axialis indistincta, centralis vero tenuiter rotundata; porus centralis raphes arcte simul, fissuris terminalibus efformantibus hamum. Striae tenuiter radiales in medio-parallelae ad apices, indistincte punctatae secundum lineam. 22·5-25 μ long., 6 μ latit., striae 26 in 10 μ.

Valves narrow, linear-lanceolate, with obtuse ends. Axial area indistinct, central area slightly rounded; Central pore of the raphe close togather, terminal fissures forming hooks. Striae slightly radial in the middle and parallel towards the ends, indistinct punctae along the line.

Dimensions :—

Length	...	...	22·5-25 μ
Breadth	...	...	6 μ
Striations	...	...	26 in 10 μ

Habitat :—Fresh-water pond near Engineering College B. H. U. In general appearance this form shows similarity to *N. spicula* (Dickie) Cleve. The present variety is distinguished by (i) it is much smaller in size (ii) in the centre a distinct space between two striae present (iii) the terminal fissures forming hook like appearances. It is therefore regarded as a new variety.

Section ... ... Naviculae decipientes Cleve

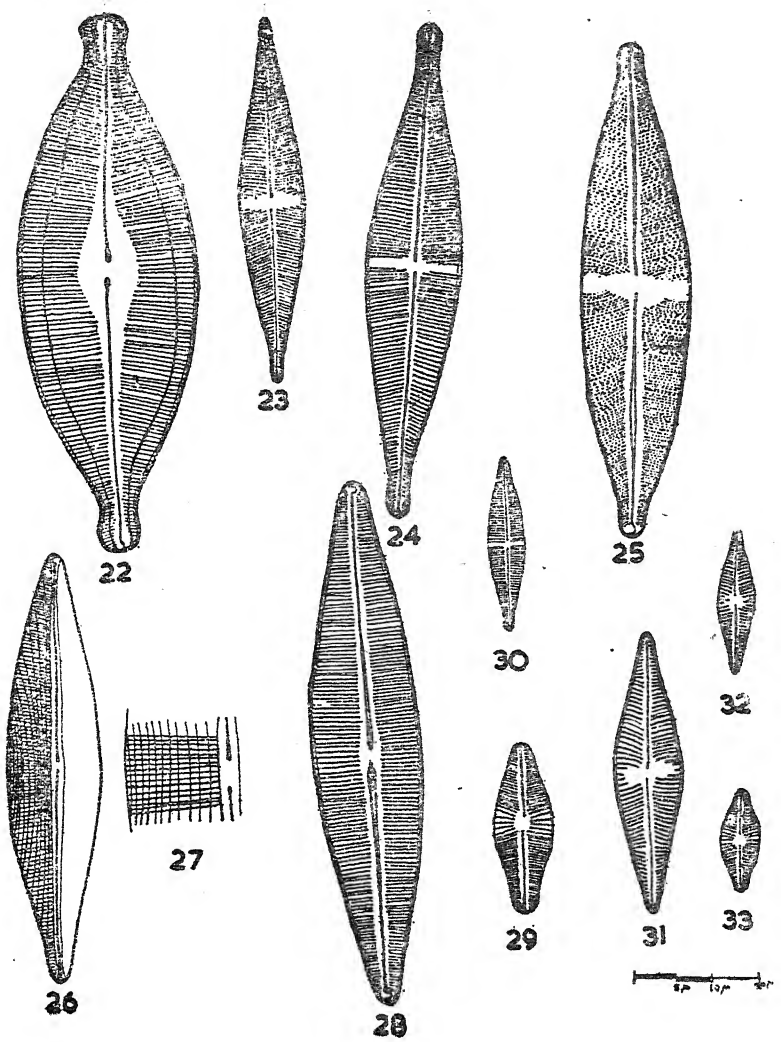


PLATE No. II

Figs. 22-33. Fig. 22. *Caloneis amphisbaena* (Bory) Cleve for. *inaicum* for. Nov. × 1500. Fig. 23. *Stauroneis anceps* Ehr. for. *gracilis* (Ehr.) Cleve × 1500. Fig. 24. *Stauroneis anceps* Ehr. var. *hyalina* Brun. & Peragallo × 1500. Fig. 25. *Stauroneis phoenicenteron* Ehr. × 1500. Figs. 26, 27. *Navicula cuspidata* Kütz. var. *conspicua* Venkataraman × 1500. Fig. 28. *Navicula cuspidata* Kütz. for. *indica* Krishnamurthy × 1500. Fig. 29. *Navicula protracta* Grun. × 1500. Fig. 30. *Navicula spicula* (Dickie). Cleve var. *minima* var. Nov. × 1500. Fig. 31. *Navicula cari* Ehr. × 1500. Fig. 32. *Navicula crystocephala* Kütz. var. *intermedia* Grun. × 1500. Fig. 33. *Navicula laterostrata* Hust. × 1500.

24. *Navicula protracta* Grun.

(Fig. 29)

Length 22·5-25 μ, breadth 6-7·5 μ and striations 18-20 in 10 μ.

Section ... ... Naviculae lineolatae Cleve

25. *Navicula cari* Ehrenberg

(Fig. 31)

Length 38-40·5 μ, breadth 9·5-10·5 μ and striations 16-18 in 10 μ.

26. *Navicula crystocephala* Kütz. var *intermedia* Grun.

(Fig. 32)

Length 12·5-22 μ, breadth 6-7 μ and striations 16-18 in 10 μ.

27. *Navicula laterostrata* Hüst.

(Fig. 33)

Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 301, fig. 521.

Valves elliptical, lanceolate, broadly rounded, with more or less capitate ends. Axial area very narrow, central area big, rounded. Striae delicate, slightly radial in the middle and closer towards the ends.

Dimensions :—

Length	...	...	15·5-20 μ
Breadth	...	...	6·5-7·5 μ
Striations in the middle		...	14 in 10 μ
Striations towards the ends		...	20-22 in 10 μ

Habitat :—Fresh-water pond behind the B. H. U. Press. The species agrees with the type very well. The striae are very delicate and were seen only with difficulty.

28. *Navicula lanceolata* (Agardh) Kütz. var. *tenella* A. S.

(Fig. 34)

Length 25·5-28·5 μ, breadth 4·5-6·5 μ and striae 14-16 in 10 μ.

29. *Navicula plancentula* (Ehr.) Grun. var. *rostrata* Mayer

(Fig. 35)

Length 32·5-36 μ, breadth 10-20 μ and striations 9-11 in 10 μ.

30. *Navicula salinarum* Grun.

(Fig. 36)

Boyer, Syn. N. Am. Diat., 1927, p. 383; Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 295, fig. 498.

Valves elliptic-lanceolate with produced subcapitate ends. Axial area narrow, central area big, rounded. Striae strongly radiate in the middle longer and shorter, and in the ends transverse.

Dimensions :—

Length	...	...	26-33 μ
Breadth	...	...	7 5-9 μ
Striations	...	...	13-15 in 10 μ

Habitat .—Fresh-water pond behind the B. H. U. Press. This specimen is not so common. The specimens agree best with the type.

31. *Navicula simplex* Krasske

(Fig. 37)

Length 32·5-34 μ, breadth 8·5-9 μ and striations 14-16 in 10 μ.

Genus ... ... PINNULARIA Ehrenberg, 1843.

Section ... ... Capitatae

32. *Pinnularia interrupta* W. Smith

(Fig. 38)

Length 31-49 μ, breadth 7-8 μ and striations 12-14 in 10 μ.

33. *Pinnularia subcapitata* Gregory var. *Hilseana* (Janisch) O. Müll.

(Fig. 39)

Length 33·5-36 μ, breadth 5·5-6 μ and striations 12-14 in 10 μ.

Section ... ... Divergentes

34. *Pinnularia microstauron* (Ehr.) Cleve

(Fig. 40)

Length 47·5-50 μ, breadth 9·5-10·5 μ and striations 10-11 in 10 μ.

35. *Pinnularia microstauron* (Ehr.) Cleve var. *Brebissonii* (Kütz.) Hüst.

(Fig. 41)

Length 49-50 μ, breadth 10-10·5 μ and striations 11-12 in 10 μ.

Section ... ... Tabellariae

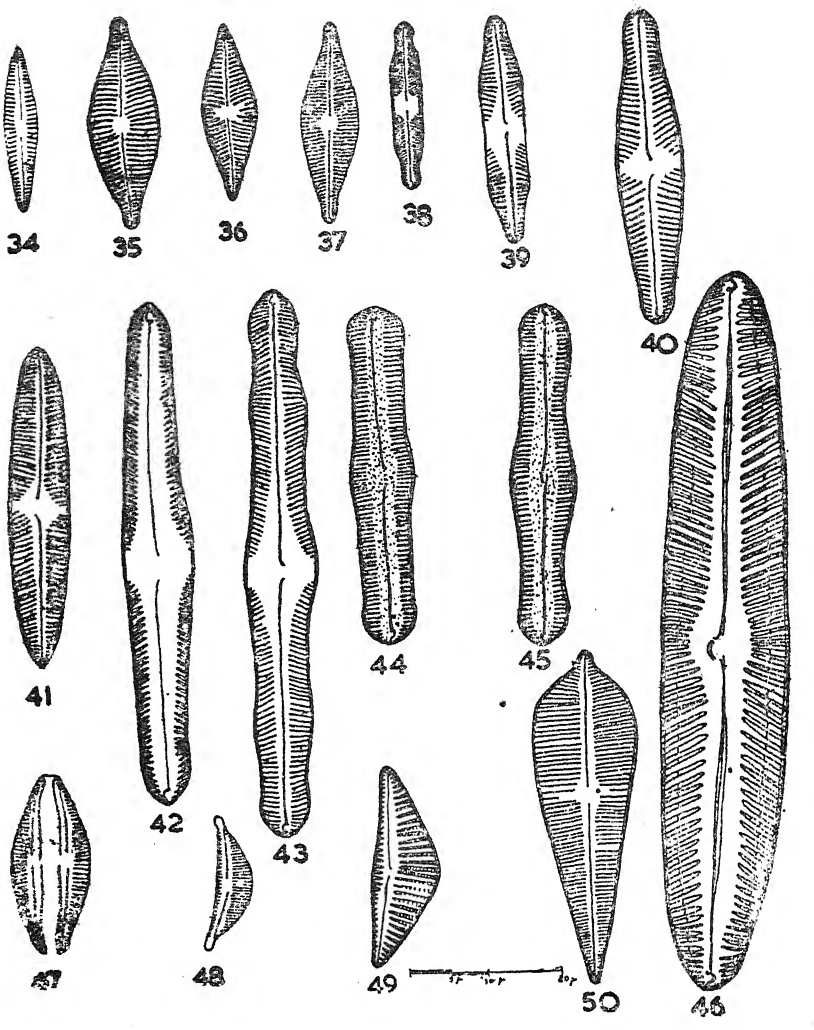


PLATE No. III

Figs. 34-50. Fig. 34. *Navicula lanceolata* (Ag.) Kütz, var. *tenella* A. S. × 1500. Fig. 35. *Navicula placentula* (Ehr.) Grun. var. *rostrata* Mayer × 1500. Fig. 36. *Navicula salinarum* Grun. × 1500. Fig. 37. *Navicula simplex* Krasske × 1500. Fig. 38. *Pinnularia interrupta* W. Smith × 1500. Fig. 39. *Pinnularia subcapitata* Gregory var. *Hilseana* (Janisch) O. Müll. × 1500. Fig. 40. *Pinnularia microstauron* (Ehr.) Cleve × 1500. Fig. 41. *Pinnularia microstauron* (Ehr.) Cleve var. *Brebissonii* (Kütz.) Hüst. × 1500. Fig. 42. *Pinnularia gibba* Ehr. × 1500. Fig. 43. *Pinnularia gibba* Ehr. for. *subundulata* Mayer × 1500. Fig. 44. *Pinnularia acrosphaeria* Brebission × 1500. Fig. 45. *Pinnularia acrosphaeria* Brebis. for. *undulata* Cleve × 1500. Fig. 46. *Pinnularia viridis* (Nitzsch) Ehr. × 1500. Fig. 47. *Amphora ovalis* Kütz. × 1500. Fig. 48. *Amphora coffeoeformis* Agardh var. *africana* Fritsch & Rich × 1500. Fig 49. *Cymbella turgida* (Greg.) Cleve × 1500. Fig. 50. *Gomphonema augur* Ehr. × 1500.

36. *Pinnularia gibba* Ehrenberg,

(Fig. 42)

Length 69-98 μ, breadth 10-12 μ and striations 11-12 in 10 μ.

37. *Pinnularia gibba* Ehr. var. *subundulata* Mayer

(Fig. 43)

Length 67·5-95 μ, breadth 10·5-12 μ and striations 10 in 10 μ.

Sections ... ... Brevistriatae

38. *Pinnularia acrosphaeria* Brebission

(Fig. 44)

Length 45-52 μ, breadth 10-11 μ and striations 10 in 10 μ.

39. *Pinnularia acrosphaeria* Breb. for. *undulata* Cleve

(Fig. 45)

Length 55-59·5 μ, breadth 10-11 μ and striations 9-10 in 10 μ.

Section ... ... Complexae

40. *Pinnularia viridis* (Nitzsch) Ehr.

(Fig. 46)

Length 93·5-107 μ, breadth 12-15·5 μ and striations 7-9 in 10 μ.

Sub-family ... ... Gomphocymbelloideae

Genus ... ... AMPHORA Ehrenberg, 1840.

Section ... ... Amphora *i. e. s.* Cleve

41. *Amphora ovalis* Kützing

(Fig. 47)

Length 26-30 μ, breadth 6 μ and striations 16-17 in 10 μ.

Section ... ... Halamphora Cleve

42. *Amphora coffeaeformis* Agardh var. *africana* Fritsch & Rich

(Fig. 48)

Length 21·5-25 μ, breadth 6-7 μ and striations 20-23 in 10 μ.

Genus ... ... CYMBELLA Agardh, 1830.

43. *Cymbella turgida* (Greg.) Cleve

(Fig. 49)

Length 30·5-41 μ, breadth 9-10·5 μ and striations 10-11 in 10 μ.

Genus ... ... GOMPHONEMA Agardh, 1824

44. *Gomphonema augur* Ehrenberg

(Fig. 50)

Length 36·5 μ, breadth 9·5-10·5 μ and striations 12-13 in 10 μ.

45. *Gomphonema augur* Ehr. var. *geninum* Mayer

(Fig. 51)

Length 25-37 μ; breadth 9-10·5 μ and striations 14-15 in 10 μ.

46. *Gomphonema parvulum* (Kütz.) Grun.

(Fig. 52)

Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10. 1930 p. 372, fig. 713*a*; Venkataraman, G., Systematic Account of some South-Indian Diatoms, Pro. Ind. Acad. Sci., Vol. 10, 1939, p. 345, fig. 121.

Valves lanceolate club-shaped gradually tepering from the middle to the ends. Axial area very narrow, central area unilateral with an isolated stigma. Striae radial, very finely punctate.

Dimensions:—

Length	... ...	13·5-20 μ
Breadth	... ...	5-6 μ
Striations	... ...	16-17 in 10 μ

Habitat :—Fresh-water pond near the Engineering College B. H. U. This species agrees best with the type. It was rare in the collections.

47. *Gomphonema varanasis* Sp. Nov.

(Fig. 53)

Valvulae clavatae, dimidio inferiore gradatim fastigato superiore vero bis constricto; apex subcapitatus, basis obtusa ad subacutum. Raphe tenuis et recta, area axialis angusta, centralis vero parva, unilateralis. Striae distinctae, tenuiter radiales, inter se plus distantes ad medium. 33·5-46·5 μ long.; 7·5-10 μ latit.; striae 10-13 in 10 μ.

Valves clavate with gradually tapering lower half and biconstricted upper half, apex gradually subcapitate, base obtuse to subacute. Raphe thin and straight. Axial area narrow, central area small, unilateral. Striae clear, slightly radial, wider apart in the middle.

Dimensions:—

Length	... ...	33·5-46·5 μ
Breadth	... ...	7·5-10 μ
Striations	... ...	10-13 in 10 μ.

Habitat :—Fresh-water pond near the Engineering College B. H. U. This form resembles *G. acuminatum* Ehr. var. *turris* Ehr. (Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 372, fig. 689) in out-line and in the number and arrangment of the striae with *G gracile* Ehr. (Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 376, fig. 702). Thus the present species cannot be included in any of the two species which it resembles in one or the other characters. The difference is marked and deserves a new specific rank, some where between the two species. It is, therefore, regarded as a new species.

Family	...	...	Epithemiaceae
Sub-family	...	...	Rhopalodioideae
Genus	...	...	RHOPLODIA O. Müller, 1895

48. *Rhopalodia gibba* (Ehr.) O. Müll. var. *ventricosa* (Ehr.) Grun

(Fig. 54)

Length 63-66 μ, breadth 21·5 μ and striations 6-8 in 10 μ.

Family	...	...	Nitzschiaceae
Sub-family	...	...	Nitzschioideae
Genus	...	...	HANTZSCHIA Grunow. 1880

49. *Hantzschia amphioxys* (Ehr.) Grun. var. *vivax* (Hantz.) Grun.

(Fig. 55)

Length 61-72 μ, breadth 7·5-8 μ, striations 18-20 in 10 μ and keel punctae 9-10 in 10 μ.

Genus	...	...	NITZSCHIA Hassal, 1845
Section	...	...	Tryblionella (W. Smith, Grun) Hüst.

50. *Nitzschia hungaria* Grunow

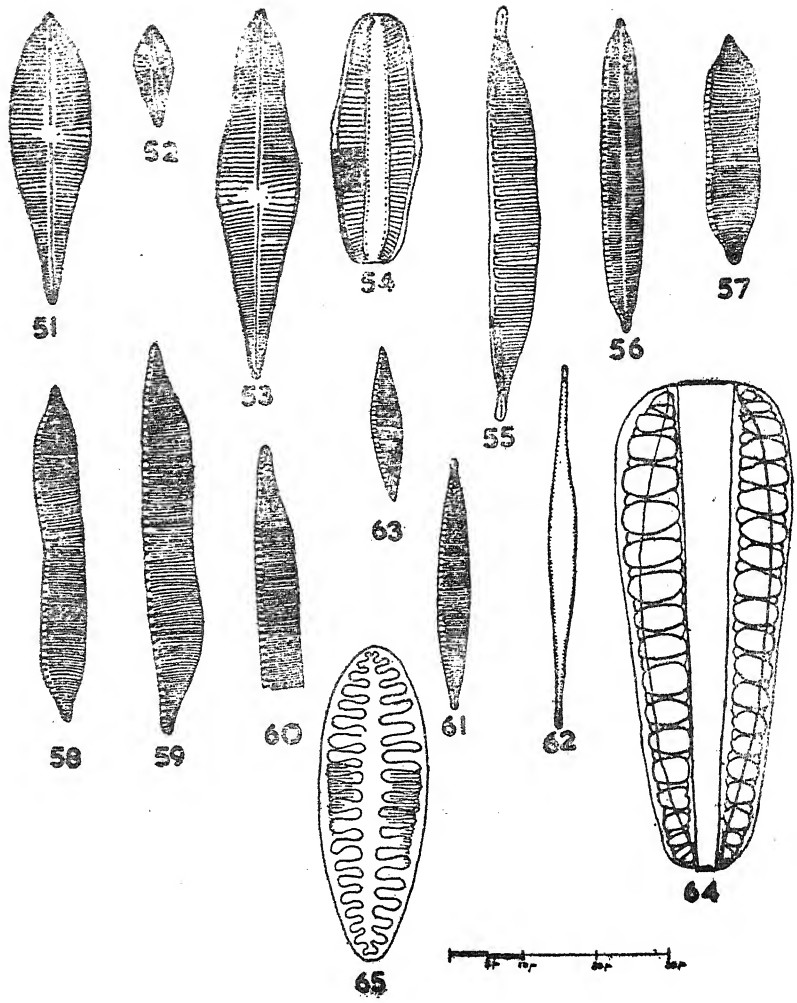
(Fig. 56)

Length 47·5-52 μ, breadth 6·5-7 μ, striations 17-20 in 10 μ, and keel punctae 9—10 in 10 μ.

51. *Nitzschia tryblionella* Hantz. var. *levidensis* (W. Smith) Grun.

(Fig. 57)

Boyer, Syn. N. Am. Diat., 1927, p. 495 ; Hüstedt, Fr., Paschers Süsswasser-Flora, Heft 10., 1930, p. 399, fig. 760.



PLATF No. IV

Figs. 51-65. Fig. 51. *Gomphonema augur* Ehr. var. *geninum* Mayer × 1500. Fig. 52. *Gomphonema parvulum* (Kutz.) Grun. × 1500. Fig. 53. *Gomphonema varanasis* sp. Nov. × 1500. Fig. 54. *Rhopalodia gibba* (Ehr.) O. Müll. var. *ventricosa* (Ehr.) Grun. × 1500. Fig. 55. *Hauntzschia amphioxys* (Ehr.) Grun. var. *vivax* (Hantz.) Grun. × 1500. Fig. 56. *Nitzschia hungaria* Grun. × 1500. Fig. 57. *Nitzschiatryblionella* Hantz. var. *levidensis* (W. Smith) Grun. × 1500. Fig. 58. *Nitzschia commutata* Grun. × 1500. Figs. 59, 60. *Nitzschia vishwanathae* sp. Nov. × 1500 Fig. 61. *Nitzschia palea* (Kutz.) W. Smith × 1500. Fig. 62. *Nitzschia acicularis* W. Smith × 1500. Fig. 63. *Nitzschia kutzingiana* Hilse × 1500. Fig. 64. *Surirella elegens* Ehr. × 1500. Fig 65. *Surirella horida* Hust. for. *minor*. Gandhi × 1500.

Valves linear with slightly concave margins. Ends wedge-shaped and rounded. A clear longitudinal fold in the middle of the valve is present. Keel very excentric. Striae clear.

Dimensions :—

Length	...	...	25-33 μ
Breadth	...	...	8-10 μ
Striations	...	...	14-15 in 10 μ
Keel punctae	...	...	11 in 10 μ

Habitat :—Fresh-water pond behind the B. H. U. Press. This is rare in the collections. It agrees very well with the type found in the literatures.

Section ... ... Dubiae Grunow

52. *Nitzschia commutata* Grunow

(Fig. 58)

Length 46-50 μ, breadth 7·5-8·5 μ, striations 21-23 in 10 μ and keel punctae 10 in 10 μ.

Section ... ... Linearis (Grun.) Hüstedt

53. *Nitzschia vishwanatkae* Sp. Nov.

(Figs. 59, 60)

Frustula ampla et robusta, lineari-rectangularia aspectu zonali, apicibus nonnihil angustis truncatisque. Valvulae lineares, robustae, binae unitae per parietes rectos vel tenuiter convexos ventrales. Latera dorsalia concava in medio, gradatim angustata ad apices, qui sunt tenuiter truncato-rotundati. Carina ventralis, excentrica. Carinae puncta brevia et unifomia. Striae robustae, lineares et uniformiter dispositae. Long, 55-114·5 μ ; latit. 5-6·5 μ striae 25-27 in 10 μ.

Frustule large and robust, linear-ractangular in the girdle-view with somewhat narrow truncate ends. Valves linear, robust united in pairs by straight or slightly convex ventral walls. Dorsal side concave in the middle, gradually narrowed at the ends, which are slightly truncate-rounded. Keel ventral, excentric. Keel punctae short and uniform. Striae strong lineate and uniformly placed.

Dimensions :—

Length	...	...	12·5-14 μ
Breadth	...	...	4·5 μ
Striations	...	...	28-30 in 10 μ
Keel punctae	...	...	11-12 in 10 μ

Habitat :—Fresh-water ponds near B. H. U. Press, as well as near Agriculture College Farm B. H. U. The specimens somewhat resembles *N. jugata* Gandhi

(Gandhi, H. P., Fresh-water diatoms of Partabgarh, J. I. B. S., Vol. 34, 1955, pp. 330-31, fig. 38) and *N. vivax* W. Smith (Hüstedt, Fr., Pascher's Süsswasser-Flora, Heft 10, 1930, p. 411, fig. 788) in the out-line and the dimensions. The present species is distinguished by (*i*) slightly convex ventral side with uniform keel punctae (*ii*) with trucate-rounded ends (*iii*) striae very close and uniformly placed. As this form appears to be distinctive, it is regarded as a new apecies.

Section ... ... Lanceolatae Grunow

54. *Nitzschia kützniana* Hilse

(Fig. 63)

Length 12·5-14 μ, breadth 4·5 μ, striations 28-30 in 10 μ and keel punctae 11-12 in 10 μ.

55. *Nitzschia palae* (Kütz.) W. Smith

(Fig. 61)

Hüstedt, Fr., Pascher's Susswasser-Flora, Heft 10, 1930, p. 416, fig. 801; Venkataraman, G., Systematic Account of some South-Indian Diatoms, Proc. Ind. Acad. Sci., Vol. 10, 1939, p. 353, fig. 146.

Valves linear to linear-lanceolate with short wedge-shaped tapering ends. Striae very delicate.

Dimensions :—

Length	...	...	35·5-40 μ
Breadth	...	...	4·5-5 μ
Striations	...	...	about 35 in 10 μ
Keel punctae	...	...	11-15 in 10 μ

Habitat :—Fresh-water ponds near Agriculture College Farm and behind the B. H. U. Press. It agrees with the type well. The striae are difficulty visible.

Section ... ... Nitzschiellae (Rabh.) Grun.

56. *Nitzschia acicularis* W. Smith

(Fig. 62)

Length 56·5-78·5 μ, breadth 3-3·5 μ and keel punctae 15-16 in 10 μ. Striae are extremely delicate and hardly visible.

Family	...	...	Surirellaceae
Sub-family	...	...	Surirelloideae
Geuns	...	...	SURIRELLA Turpin, 1828

57. *Surirella elegans* Ehr.

(Fig. 64)

Boyer, Syn. N. Am. Diat., 1927, p. 537; Hüstedt, Fr., Pascher's Süsswasser-Flora, Haft 10, 1930, p. 440. figs. 858-859.

Frustules in the girdle-view wedge-shaped. Valves narrowly or broadly ovate, rounded at one end and acute at the other end. Central space narrow, lanceolate. Coastae broad.

Dimensions :—

Length	...	...	54·5-74·5 μ
Breadth	...	...	22·5-26 μ
Striations	...	...	8-10 in 10 μ
Coastae	...	...	15-16 in 100 μ

Habitat :—Fresh-water pond near the Agriculture College Farm B. H. U. This form agrees with the description and figures given in the literatures. It is smaller and very common in this pond.

58. *Surirella horida* Hüst. for. *minor* Gadhi

(Fig. 65)

Length 54·5-74·5 μ breadth 22·5-26 μ, striations 8-10 in 10 μ and coastae 15-17 in 100 μ.

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STUDIES IN THE HYDROPHYTES OF GORAKHPUR :

OBSERVATIONS ON THE ANATOMICO-PHYSIOLOGICAL CHARACTERS OF ASTERACANTHA LONGIFOLIA NEES.—II

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[Received on 30th December, 1959]

INTRODUCTION

Asteracantha longifolia Nees., has also been described as *Hygrophila spinosa* T. Anders. It is met with in ditches and swamps and is a stout herb with erect stem mostly branched in water, otherwise simple or less branched. Stem is cylindrical, and clothed with hairs. Leaves are simple upto 6 inches long, opposite, sessile and lanceolate. Flowers are bilipped and purple coloured and about an inch long in dense axillary leafy clusters. Plants come up by August-September and flower till October-November.

The plants show various morphological variations depending upon the changing habitat. They show evolutionary morphological and anatomico-physiological adaptations, which show that aquatic species of *Hygrophila* have given rise to *Asteracanthas* as has been suggested by Santapau [1951]. "It [*Hygrophila quadrivalvis*] helps to make the perfect transitional series from the smaller and simpler *Hygrophila* to *Asteracantha*". This statement is borne out by the following morphological variations noticed in submerged and land forms, [Fig. 1 and Plate 1].

Submerged form	*Land form*
1. Stem very thick and soft due to aerenchyma.	1. Stem not very thick and hard.
2. Stem is more green and less purple coloured.	2. Stem is more purple coloured.
3. Many roots are given out in form of stilt upto two or three nodes from the base, which also turn green.	3. No such adventitious roots are given out at the nodes in the ordinary land forms.
4. Plant parts are less hairy.	4. Plants are profusely hairy and rough.
5. Internodes are long and hence submerged plants are taller.	5. Internodes are short and hence land forms are shorter.
6. Leaves are less crowded but comparatively longer.	6. Leaves and bracts are very much crowded on each node and shorter.
7. No spines are present on the nodes.	7. Six spines at each node are present, which are yellow coloured and very prominent.

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MATERIAL AND METHOD

Asteracantha longifolia is not a very well distributed plant in this area. The material was collected near the Rapti river and from a roadside pond in a swamp on the way to Tinkonia forest range. The anatomico-physiological aspect of the plant has been studied from the fresh material collected on different excursion trips.

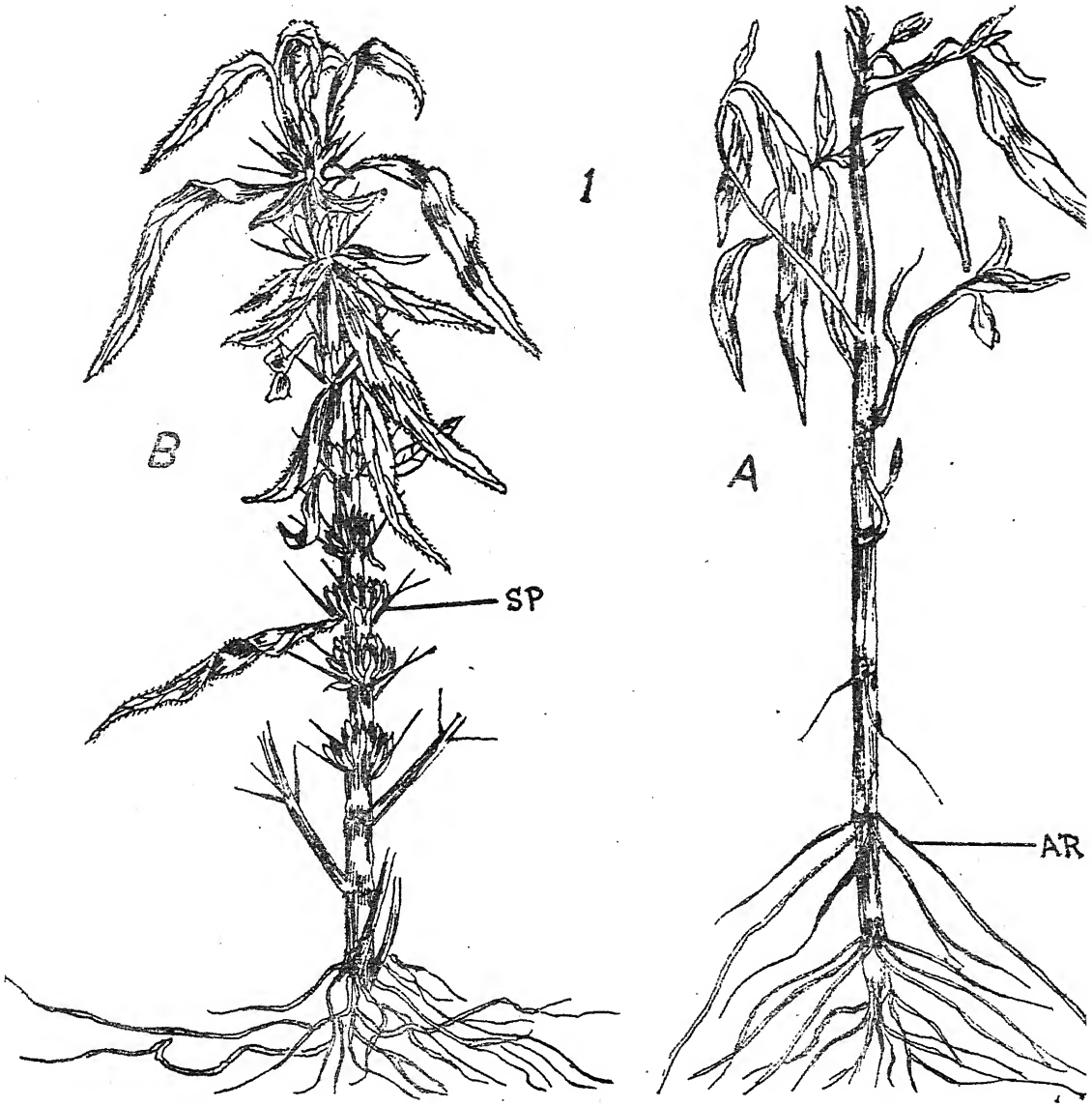


Fig. 1. Submerged [A] and the Land [B] forms of the plant [Approx. 1/4 Nat. Size].

Free-hand sections were cut from fresh material as hydrophytes present inherent difficulty in microtomy, a process involving a much longer time. Cystolith in the epidermal cells presented difficulty in sectioning the material. Epidermal peelings were also mounted and observed.

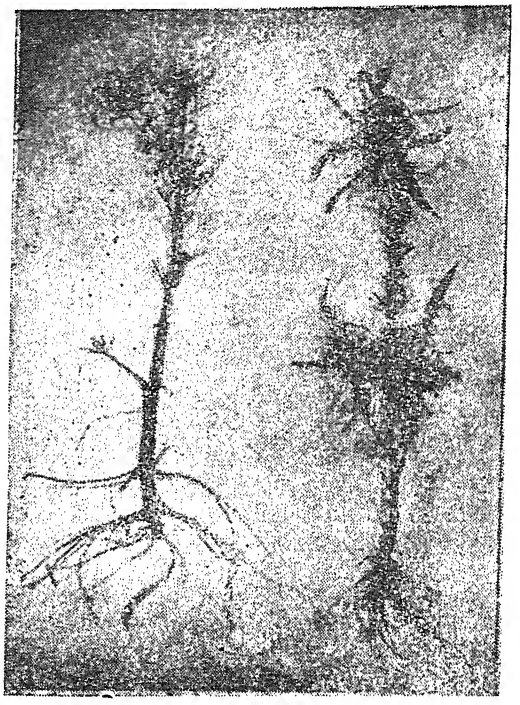


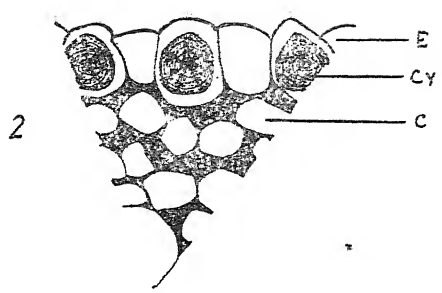
Plate 1. Photograph showing the submerged and land forms of the plant, with characteristic differences.

DESCRIPTION

The anatomy of the stem and later of the root and leaf is described below :—

Epidermis. It is made up of a single layer of thin walled cells, without any distinct cuticle even in the land forms [Fig. 2]. The vacuoles of cells in the land forms are usually filled with anthocyanin solutions which are missing in submerged forms. Solitary elongated cystoliths are abundant in the epidermal cells. These cells are slightly larger and more triangular than other epidermal cells. Cystoliths are elongated, with one end pointed, and are more in land than in aquatic forms [Fig. 3]. Stomata are caryophyllaceous raised above the level of ordinary epidermal cells. The collenchymatous hypodermis is broken below the stomata by a few chlorenchymatous cells [Fig. 4]. Hairs are uniseriate, multicellular with a bulbous pedestal base [Fig. 5]. Peltate multicellular glands are of rare occurrence in the submerged forms.

Cortex. The outer cortex consists of collenchyma, which is well developed in the submerged as well as land forms, with the difference that the cells are larger and thinner, and poorly developed in the submerged forms. The collenchymatous



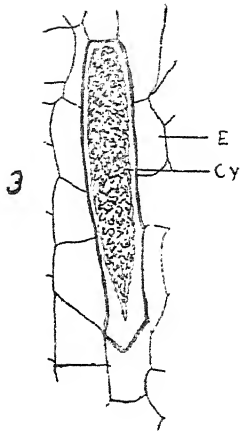


Fig. 2. Part of the T. S. of stem showing the epidermis with no cuticle and cystolith present in it. × 320.

Fig. 3. Epidermal peeling showing the elongated cystolith with one end pointed. × 200.

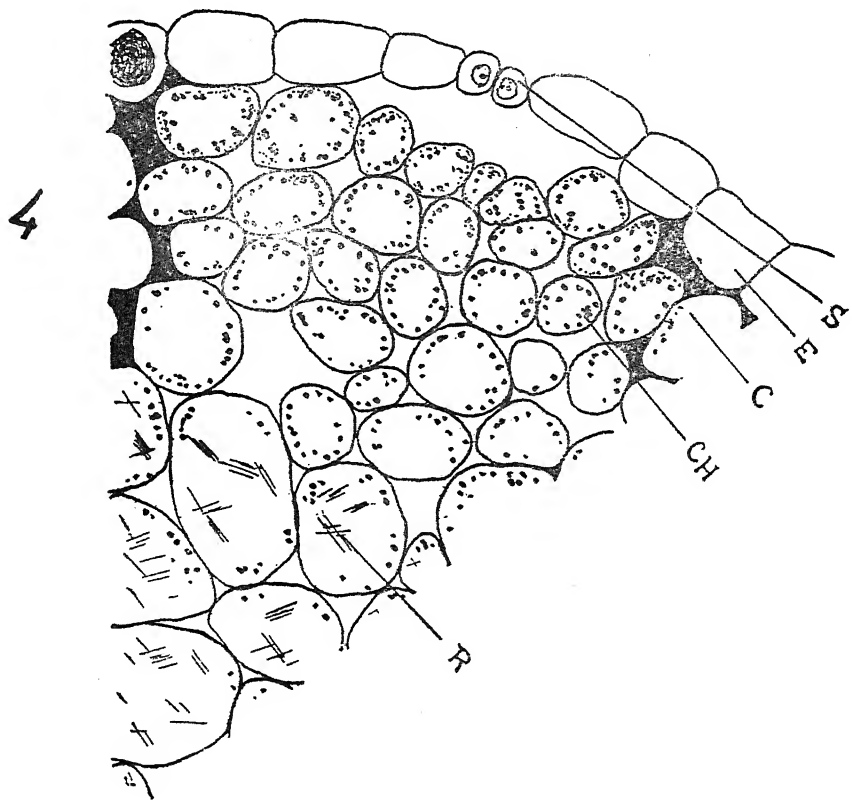


Fig. 4. Part of the T. S. of stem showing collenchymatous hypodermis which is broken by a few chlorenchymatous cells below the stomata. × 200.

continuity of cells is broken by a group of chlorenchymatous cells below the stomata [Fig. 4]. The submerged stem also possesses a few chloroplasts in the collenchymatous layer abutting the epidermis. The inner part of the cortex is made up of aerenchyma with very large air spaces in the submerged and fewer spaces in the land forms [Figs. 6-7]. The cortical cells are radially elongated in the submerged

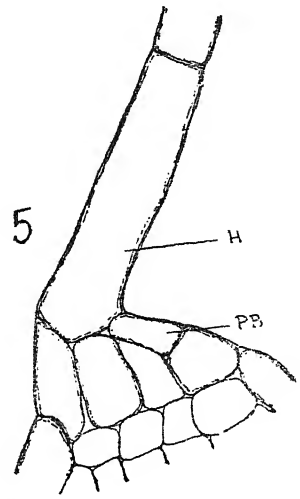
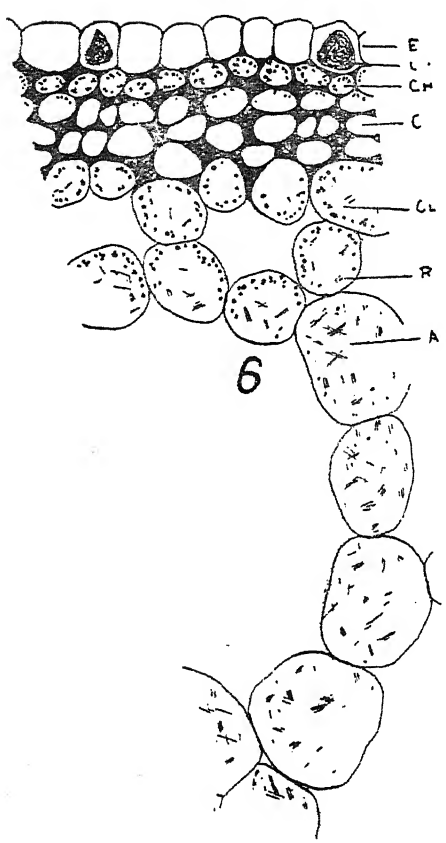
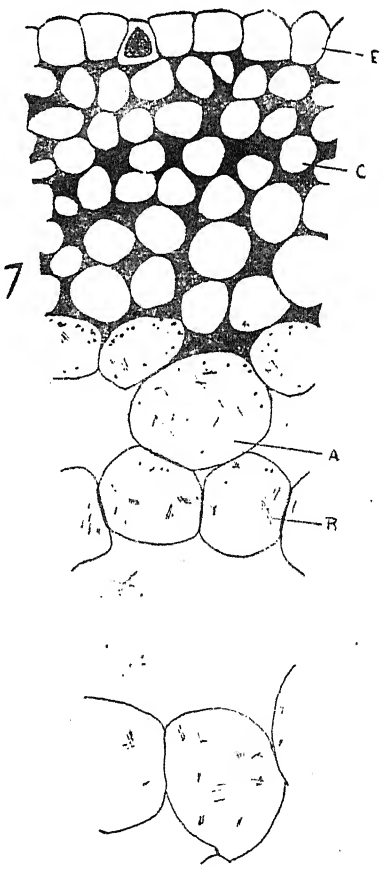


Fig. 5. Multicellular uniseriate hair with pedestal base. × 150.

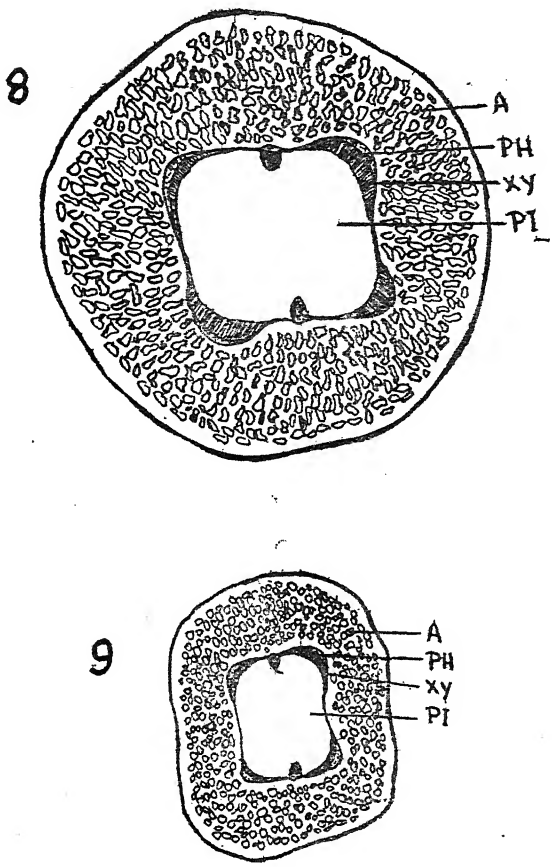




Figs. 6—7. Part of the T. S. of Stem in aquatic and land forms, showing the difference in collenchyma and aerenchyma etc. in the two cases. × 240.

and roundish in the land forms. The cortex is therefore much broader in the former than in the later [Figs. 8-9]. The layer of parenchyma cells lying just below the collenchyma abounds in chloroplasts which decrease in cells from the peripheral to the central region. This layer is also prominent due to the presence of anthocyanin, which as has already been stated is not present in the epidermal cells of the submerged forms. The innermost part of the cortex consists of 1-2 layers of parenchyma cells abutting against the endodermis.

The endodermis is very distinct as a wavy starchy sheath. Starch grains are very prominent [Fig. 10].



Figs. 8—9. T. S. of stem in aquatic and land forms [semi-diagramatic]. × 12 app.

Perieycle. The nature of the pericycle fibres are controversial [Eames and MacDaniels, 1947 ; Metcalfe and Chalk, 1950 ; Esau, 1955]. So this term used here is adopted for its positional rather than its anatomical value. The cells below endodermis consists of isolated fibre cells of thin walled parenchyma cells and thick-walled sclerenchyma fibres, [Fig. 10].

Vascular System. The stem is cylindrical in the submerged form and rectangular in the land form in outline, but stele in both the forms is rectangular or rather square. The vascular system consists of separate vascular bundles which are joined together by the formation of interfascicular cambium developing in due course. The secondary growth is normal and as a result an appreciable amount of secondary wood is formed. The phloem is present which includes a number of patches which are fibrous [Fig. 10].

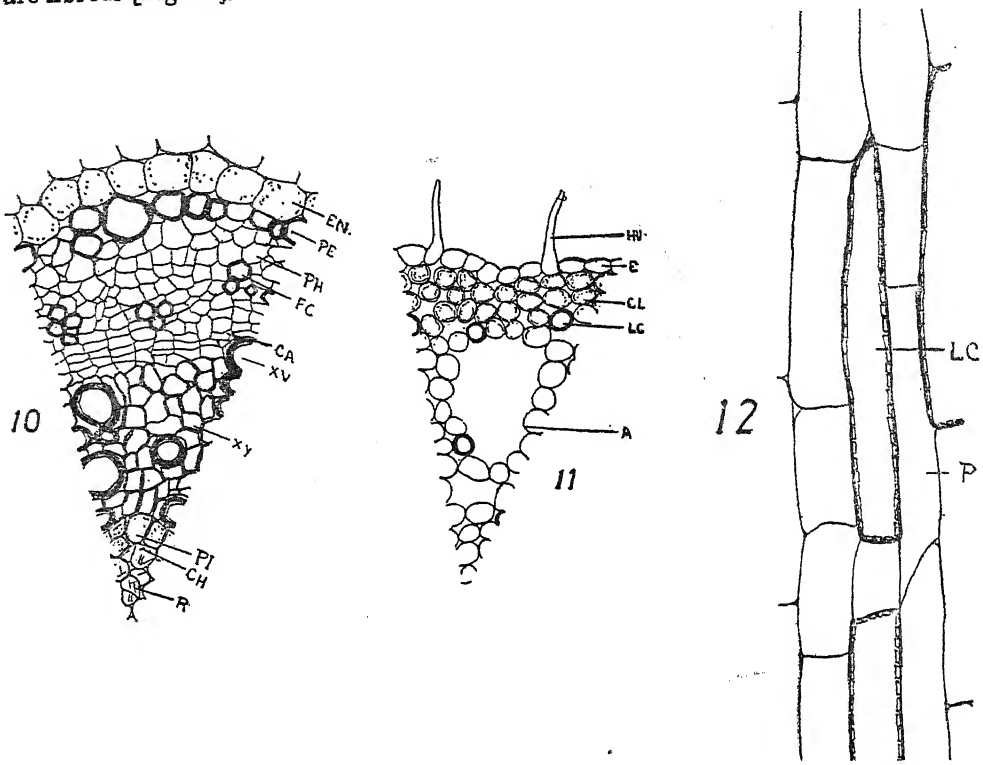


Fig. 10. Part of the T. S. of stem showing the endodermis with starch grains, pericycle followed by phloem having a number of fibrous patches, lignified tissue of xylem elements, and the pith cells near protoxylem points containing chloroplast. × 200.

Fig. 11. Part of the T. S. of adventitious root showing chloroplst, in hypodermis, and scattered lig nified cells in cortex. × 50.

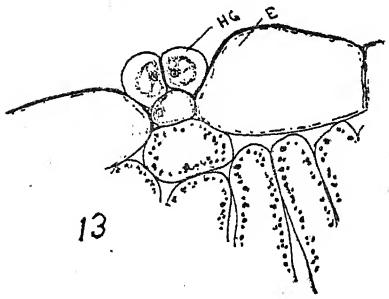
Fig. 12. Part of L. S. of adventitious root showing lignified cells with pitted thickening on their walls. × 80.

Pith. It consists of thin walled parenchymatous cells. Pith cells surrounding the protoxylem points in the vascular bundles also contain chloroplasts. There are no air spaces in the pith, or any stored starch, but raphides are plentifully present in the cells.

Roots. Roots come out from nodes and form stilts in submerged forms (Plate 1). The epiblema is covered with a large number of short unicellular hairs which are quite irregular cells. The hypodermis consists of 2-3 layers of parenchyma having chloroplasts. The main cortex is aerenchymatous with prominent and radially elongated roundish cells traversing from the centre to the periphery [Fig. 11]. In

the innermost part of the cortex are very distinct mostly solitary lignified cells with pits on the walls. These cells are rare in the outermost part of the cortex and none in the middle aerenchymatous zone. The cells are longer than broad and sometimes very long and distinct from the other neighbouring cortical cells, [Fig. 12]. Such cells are absent from the stem. The endodermis and pericycle are quite distinct for ming circular layers all around. No fibres are present in the pericycle. The roots show a tetrarch to pentarch stele. The secondary growth takes place quite early and the resulting wood formed is normal.

Leaf. The leaf is dorsiventral but the mesophyll tissue is not much differentiated dorsiventrally. Hairs are both of the glandular and non-glandular types. Glandular hairs are very few and small. The basal cell of the glandulars hair is much below the level of ordinary epidermal cells ; so that the tip of the hair is levelled with the exterior surface of the epidermal tissue [Fig. 13]. Stomata equal on both sides are of caryophyllaceous type. Collenchyma which is almost two layered thick is present on the lower side. The petiole exhibits a single arc-shaped vascular strand. Subsidiary bundles are present towards the adaxial side of the petiolar wing. Air spaces are weakly developed on both the sides in the mid-rib



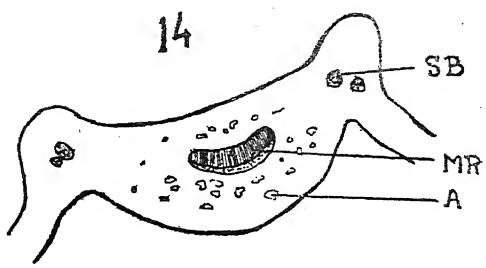


Fig. 13. Glandular hair. × 320.

Fig. 14. T. S. of petiole showing the mid-rib with a single arc-shaped vascular strand with subsidiary bundles [semi-diagrammatic]. × 12 app.

portion of the petiole [Fig. 14]. The endodermis with starch grains in the cells is distinct all around but it is not prominent on the lower side. Fibres are present in the phloem in the vascular strand.

No case of heterophylly was observed in the plants collected by the author as has been reported by Dutt [1952].

ANATOMICO-PHYSIOLOGICAL ASPECT

Asteracantha longifolia is primarily a marsh plant but grows very well when it emerges out of the water and even in the moist land or mud brought about by changes in the weather conditions. A number of morphological and anatomical changes take place as the habitat becomes comparatively dry in order to equip the plant to carry on the physiological processes in its new set up.

The aerenchymatous stem and adventitious roots whieh come out from the lower nodes for storage of gases and keeping the plant buoyant in water turn green when the plant grows in moist conditions in response to photosynthetic requirements.

The plants in drier habitats develop strong root system and are devoid of adventitious roots.

The greater development of protective and conductive tissues take place when plants take to land habitat and hydrophytic characters undergo a gradual reduction. The combined presence of hairs and six nodal spines are characteristics of both types of plants, aquatic and terrestrial; a greater development of these characters take place as the plant takes to a land habitat and a reduction occurs when the plant thrives in an aquatic surrounding. The amphibious nature of *Asteracantha longifolia* is confirmed by the physiological anatomy of the plant described above.

The family Acanthaceae to which *Asteracantha longifolia* belongs is one of the advanced families in the gamopetalous dicotyledons. So the presence of such a member in the family is certainly noteworthy from the point of view of evolutionary sequence. It may present a stage in ecological evolution indicating that it has gradually taken to a land habit from aquatic surroundings. In fact it may possibly be one of the links between the aquatic members of the family and those that have taken to terrestrial life during the course of ecological evolution in response to the changed environmental conditions. The capacity of *Asteracantha longifolia* to grow as water and also as land plant, the presence of spines at each node and other anatomico-physiological changes which terrestrial habitat brings about show as to how successfully it has taken to such an environment.

DISCUSSION

That *Asteracantha longifolia* Nees., is capable of taking to a terrestrial life, is clear from the foregoing account. The resulting environmental changes bring about morphological variations necessary for such a life. The physiology of the tissues also change with the changing anatomy of the plant parts. The following points in the plant are characteristic from anatomico-physiological view point:—

1. The poor development of cuticle and the presence of comparatively fewer hairs in the submerged portions of the stem together with the chlorenchyma abutting at places against the stomata, would possibly lead to an increase in surface absorption and photosynthetic activity.

2. The collenchyma is feebly developed in plants growing in water in comparison to its greater development in plants on land, which may be correlated with the hydrophytic requir ements of the plants growing in water, and the necessity of greater support required for the terrestrial ones.

3. The air spaces become enlarged and radially elongated in aquatic forms but become smaller and strengthened with roundish cells in land forms. The large air spaces in the cortex would facilitate aeration necessary for an aquatic environment but they loose their importance in land forms. These help also in keeping the plants buoyant in water.

4. The pericycle is fibrous in patches which is natural to an aquatic environment. The presence of fibres in phloem, however, cannot be correlated with the aquatic habitat.

5. The stele is comparatively reduced in aquatic forms but show normal secondary growth.

6. The roots show solitary fibrous cells with pitted walls scattered in the cortex. Their physiological significance is well brought out by the fact that roots function as stilt for the plant, but the cortex is all lacunar and hence their presence

affords some sort of mechanical support. These cells have also been reported in the stem of *Hygrophila quadrivalvia* Nees., by Mirashi but no physiological function has been attributed to them [Mirashi, 1957]. The author in the present case did not observe such cells in the stem.

7. The development of chlorophyll in the cortex of roots would help in photosynthetic activity.

8. Starch grains have nowhere been observed; this has also been reported by the author in the case of *Jussieua repens* [Sen, 1959].

From the foregoing discussion it becomes evident that with the consequent drying of the habitat, the plant very successfully takes to terrestrial life and a number of morphological and anatomico-pysiological changes take place to equip it better to face the changed environmental conditions.

SUMMARY

The anatomico physiological characters of *Asteracantha longifolia* Nees., have been studied in this paper. The plant grows in water or swamps but it takes to terrestrial life when environmental conditions change. In the latter condition adventitious roots which are green in colour in an aquatic environment, disappear. These have fibrous cells in cortex to give mechanical strength to the plant in an aquatic environment. Six nodal spines together with profuse growth of hairs on aerial parts of the plant appear in response to the changed dry environment, in which number of other changes have also been observed in the plant from the anatomico-physiological point of view. These changes would possibly equip it better to face the dry environmental conditions.

ACKNOWLEDGMENT

It is a great pleasure to express my indebtedness to Dr. U. N. Chatterji of the Gorakhpur University for help and guidance during the course of this study and for his critically reading the manuscript. Thanks are due to Prof. M. O. Varkey, Principal, St. Andrew's College, Gorakhpur, for providing research facilities to the author, and to his colleagues and students for their help in various ways. Grateful thanks are due to Dr. B. C. Kundu, Director, Jute Agriculture Research Institute, Barrackpore, Bengal for the literature he sent to the author.

ABBREVIATIONS USED

A—Aerenchyma,
AR—Adventitious roots,
C—Collenchyma,
CA—Cambium,
CH—Chloroplast,
CL—Chlorenchyma,
CY—Cystolith,
E—Epidermis,
EN—Endodermis,
FC—Fibrous cells,
H—Hair Uniseriate,
HG—Hair Glandular,
HU—Hair Unicellular,
LC—Lignified cells,
MR—Mid-rib,
P—Parenchyma,
PB—Pedestal base,
PE—Pericycle,
PH—Phloem,
PI—Pith,
R—Raphides,
S—Stomata,
SB—Subsidiary bundles,
SP—Spines,
XV—Xylem vessel,
XY—Xylem elements.

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STUDIES ON GALL MIDGES (*ITONIDIDAE*: DIPTERA)

By

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The long felt need for consolidating information regarding the gall midges, is dealt with in this paper. The information is contained under three heads viz. Collecting, Preserving and Studying. Different methods of collecting midges are described in detail. The special process to be adopted in preserving these delicate insects is also given in detail. Different lines on which the study of midges can be carried out is dealt with in detail.

This group has been little known in our country, probably beceause of the manifold difficulties involved in the study of these minute and delicate insects. Their very small size and peculiar habits of developing inside the gall and sometimes pupating inside the soil, make it extremely difficult to collect or rare the midges. In addition, a considerable number of these midges do not at all produce galls: some live on dead and decaying vegetable matter, others attack the scale insects, plant lice, gall midges, etc. Thus, the type of 'Home' and the mode of 'Life' of these insects is, to a very great extent, responsible for this group, though important, so much neglected in our country. In the absence of any authentic and published matter on the preliminaries of the studies of these delicate insects, gall midges, except for the occasional notes of Barnes (1946), Mani (1948) and Pritchard (1951) and hints in general for Diptera by Beirne, et. al. (1955), Colas (1956), Davies (1954), Harold Oldryod (1958), Oman and Cushman (1948), Parmenter (1951), Schenk, et. al. (1956), Smart (1940), and Wagstaffe & Fidler (1955) this note, is hoped to be of immense help to the beginners in this field and will meet the needs of those who have been constantly asking for information.

Study of gall midges is broadly divisable into three sections: COLLECTING, PRESERVING and STUDYING.

COLLECTING

Gall midges can be collected nearly all through the year (A) at light or (B) reared from their galls.

A. *Collections at light*: Innumerable number of midges are attracted to light. It is always advantageous for a beginner to possess midges collected at light, in large numbers, as these will not be less valuable than the reared midges, which are usually in smaller numbers. Ordinary household lights also attract midges but blue and flood lights are more suitable. Bathroom lights are also equally effective in attracting the midges (Harold Oldroyd, 1958). Separating the midges from the closely allied ganats, also attracted to the lights, may not be easy for an untrained eye. However the absence of the plumose antennae and the tibial spurs in the midges can not be left un-noticed. Besides, the reduced wing venation is an unmistakable guide to distinguish these insects from their allies. Further the experience of one or two attempts will make the collection more easy than any amount of theory (Fig. 1).

One of the easy methods of collecting midges at light is with a small brush dipped in 70% alcohol: Take a little quantity of alcohol in a small tube (2 × 0·75") and a brush with plenty of bristles (the one used by painters will also be very suitable). Dip the brush in spirit and just touch the flying or resting midge with this wet brush. The midge immediately gets stuck up to the brush. Bring the brush slowly in to the tube and leave the midge safe in the alcohol. It is very easy to collect midges resting or flying, in the close vicinity of lights, in this manner.

Light traps can be employed for collecting the midges. This method as well as collection of midges by sheeting (Spreading a white sheet of cloth with a light in the middle) are not very useful, as the separation of the midges from other small insects, collected along with them, will not be very easy.

B. *Rearings from galls* : Gall Midges can be very conveniently reared from their galls. Knowledge of the host plant of the midge is very helpful in the study of midges. It will avoid unnecessary confusion and at times duplicacy of work, because the same midge collected at different places and seasons may present slightly dissimilar characters. Such a dissimilarity may lead to incorrect study resulting in multiplication of species. Further, important biological notes can always be recorded by rearing the midges on the plant. Midges from the galls on the plants can be reared in the following ways :

On the plant : Midges are best reared by engulfing the galls. This is best done by selecting one or more suitable mature galls (that can be easily approached). After such a selection is made, engulf this part of the plant (as small a part as possible) either in (*a*) glass cylinders or (*b*) with cellofin paper.

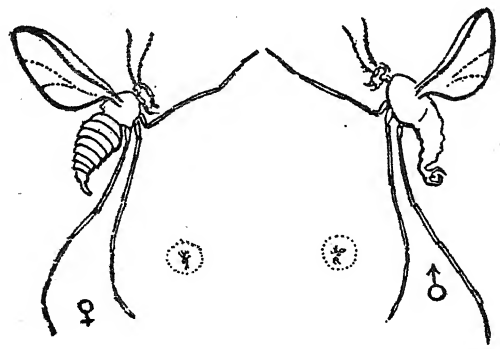

Fig. 1

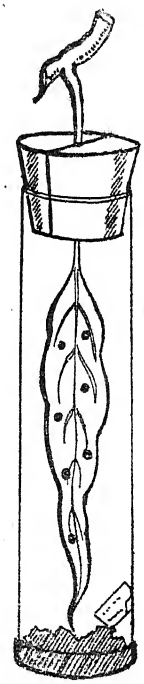

Fig. 2

Glass Cylinder Method : (Fig. 2) Take a glass cylinder normally of the length between 6 and 12 inches and of the diameter of one to two inches. As far as

possible, this glass should be of unbreakable quality and also light. One end of the cylinder should preferably be rimmed. Cover this rimmed end of the cylinder with muslin cloth, holding it fast to the cylinder with a rubber band. The muslin cloth is sufficiently porous to allow the engulfed part of the plant (leaf, etc.) to respire freely. The outer end of the cylinder is to be fitted with a cork of light material. This cork is to be split into two and a small grove made in the middle of the two parts to allow the plant (leaf petiole, tender twig, etc.) to fit in nicely. Slip the selected part of the plant (bearing galls) into the cylinder and close it with the cork, leaving the necessary data inside the tube on as small a paper as possible. The gall is now safe inside the cylinder, getting its usual nourishment as before (as it is not sparated from the plant). Tie the glass cylinder to another and more permanent part of the plant as a caution against its falling off. Go on recording the notes of day to day progress. Midges that emerge will be flying against the walls of the glass cylinder and when these are sufficient in numbers, disconnect the galled part from the plant. Then bring the cylinder into the laboratory. Remove the rubber band and only a part of the muslin cloth. This will enable the midge to be collected in the usual manner. Label the midges and the tube with all the necessary data and keep it in the store.

This is a very convenient method of collecting midges from galls on such parts of the plants as the leaves, terminal buds and small branches. It cannot however be employed with the same convenience for the galls on the branches or stem which are more woody.

Cellofin Paper Method : Cellofin paper can be employed in the place of the glass cylinder, with much more ease and convenience. Take cellofin envelopes of moderate size and engulf the effected part of the plant. Seal the ends of the envelope with a string or by pasting, after leaving the data inside as usual. Watch the progress and when sufficient number of midges emerge, cut the part of the plant and collect the midges as usual in the laboratory. Keep the collection in the store after noting the necessary data.

It is not always necessary to use the cellofin in the form of envelopes only. Sheets of cellofin can be losely rolled aroud the gall, leaving sufficient space and both the ends tied with twine.

This method is specially suitable, where the effected part of the plant cannot be fitted in glass cylinders and is more suitable for the woody galls on branches and stems. Inspite of its advantages, this method suffers from certain defects. The respiration of the engulfed part of the plant is effected; birds and squirrels tear off the cellofin and rain spoils the envelope, specially if gum is used. All these defects can be overcome, if thin wiregauge is used instead of cellofin.

In the laboratory : Both the above mentioned methods are not suitable in the case of midges that pupate in the soil. Such of the midges that pupate underground are to be reared in or collected from the breeding cages in the laboratory.

Breeding cage method : This method is suitable nearly in the case of all types of galls and specially those that pupate in the soil. Breeding cages are of two types: The Belljar type and the Batteryjar type.

Bell-jar type breeding cage : This method involves separating the galled part from the mother plant and allowing the galls to grow further in the laboratory. Separate the galled part of the plant by bending the galled branch, etc. in to a

trough of water and then cutting it under water. Care should be taken to keep the cut end of the galled twig in the trough always under water. Then bring the whole trough into the laboratory still keeping the cut end under water. Fit a rubber tube to the tap in the laboratory and allow the water to flow through it until all the air in the rubber tube is driven out. Then regulate the flow of water to a moderate strength in the tube. Now connect the rubber tube (under water) to the cut end of the twig making sure that all is water tight. Thus maintain a constant and steady supply of water to the twig by regulating the flow. Keep a layer of moistened sand or fine soil (about half or one inch thick) on a table or stool near

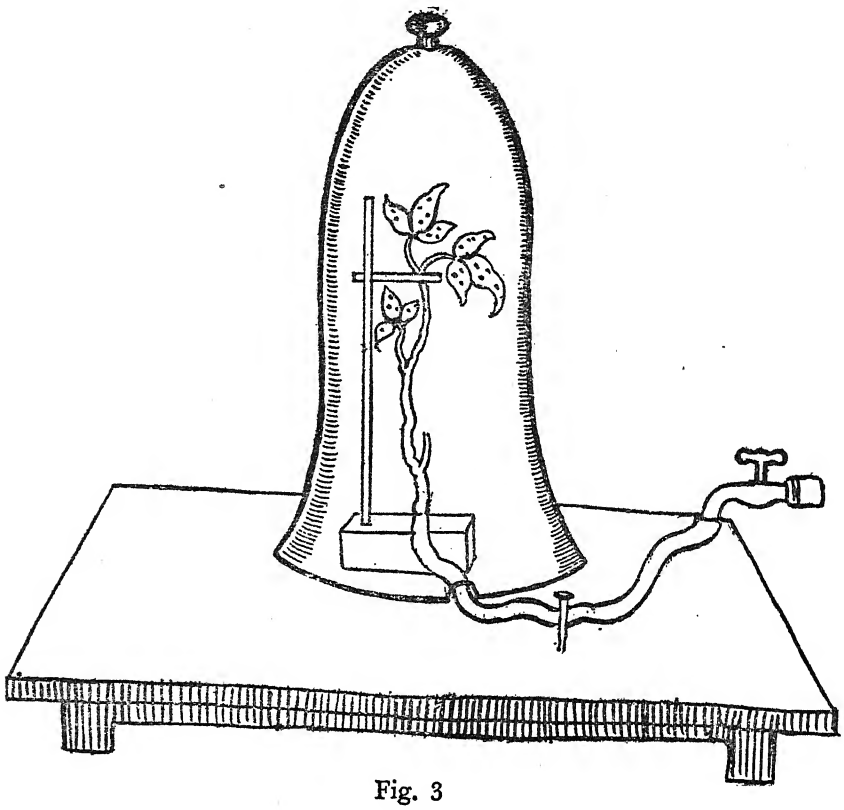

Fig. 3

the tap. Fix the twig to a stand as shown in figure 3. Keep the stand on or near the sand and cover the plant with a bell-jar. This ensures the constant supply of water to the plant which helps the galls to have their normal nourishment. The layer of moistened sand will be utillised by the mature larvae that drop down, to pupate in the soil. The bell-jar will trap the emerging midges. The midges can be colleted in the usual manner with a wet brush.

This method is of great importance to the worker as it will enable him to record few points of interest like the sex ratio at the time of emergence, in addition to the general rearing and collection. Furtner the life history of the midge can also be studied, as the fresh midges that are emerging will deposit the eggs on the leaves of the host plant.

Batteryjar type breeding cage : This method is less troublesome and consequently less effective than the one described above.

Take a batteryjar, either rectangular or circular and keep a layer of moistened sand in it. Collect mature galls from the infected plant and keep them in the jar. Cover the jar with a muslin cloth and hold it fast with a rubber band, leaving the data inside as before. Go on observing the emergence of midges and collect them as before. In this method the selection of the material is important because tender galls will dry off quickly resulting in the failure of the experiment. Only fully ripe or mature galls will liberate the fullgrown larvae that can pupate in the sand provided. Care should be taken also not to select over-mature galls, from which most of the larvae have already pupated in the soil.

This method is specially advantageous to collect midges that pupate underground and can also be employed with convenience and efficiency for rearing any gall-forming midge.

Chimneys and other Turtox cages can also be used instead of the bell-jar.

Nets and other similar appliences are not suitable for collecting these delicate insects.

PRESERVING

Like any other small insect gall midge can be preserved either on pins and card tips or in alcohol. Thus there are two types of preserving the gall midges: Dry preservation and Wet preservation.

DRY PRESERVATION

The minute size of the midges and their extremely fragile antennae and legs, coupled with the excessive heat of our country does not prove very suitable for the midges to be pinned and preserved dry. However some of the larger specimens like the *Misospatha, Asphondylia, Pachydiplosis, Orseollia* and a few others can be pinned and preserved in the midge-tubes.

Select very fine pins, not the ordinary pins, but the silver quoted or nickle painted entomological pins. Invert the midge and drive the pin with the help of the entomological forceps in between the mid coxae, taking care that the pin does not project on to the thorax, the other side. Fix this pin on the card or a piece of pith. Write all the data on a small piece of paper and pin this also along with the midge. Specimens pinned in this manner are not to be kept in the general insect store boxes, because of the danger of the mould attacking the midges rendering them useless for study. Thus these midges are to be preserved in the midge-tubes. If for any reason the midges so pinned are kept in the general store, it is desirable to preserve duplicate spicimens in alcohol also.

Triangular pieces of card or celluloid can also be employed in preserving the midges. Here the whole insect will have to be glued with the help of any adhesive. But specimens preserved on the card tips or celluloid bits also suffer from the same defects as those pinned. Further the adhesive used in fixing the specimens also hastens some chemical change and renders the midge not suitable for scientific study.

This method is not useful in general but is very convenient for preserving special type of midges like *Charidiplosis* and *Lasioptera* whose body and wings are covered with scales.

WET PRESERVATIONS

Midges are best preserved for purposes of scientific study in alcohol of 75% stength, with a little amount of glycerine added to it, to keep the material rather soft. The use of the small specimen tubes of the size 0·5 × 2·00" is the best for this purpose.

Other preservatives like the 4% formaldehyde, Bele's fluid may be used but not with the same success.

Care should be taken not to preserve a large number of midges in the same tube and not to use any fibrous material like cotton for plugging the tube. It is better to use tissue paper rolled in the form of a cork for the tube. To avoid evoperation of the lipuid, all these small tubes containing midges and corked with tissue paper are to be stored in larger bottles containing alcohol.

STUDYING

Study of the midges involves many difficulties like the suitable material, availability of litarature, etc. Empty the midges from the small tube in to a cavity block and select our one or more specimens of each sex from the lot before you, observing them under a Bionocular microscope. Take a small brush and separate the midges, at least one male and one female, keeping in view that these are as complete as possible. Replace the unwanted material back in the tube. Keep the selected midges in alcohol in the cavity block for dehydration. Take care that the delicate antennae, wings and legs of these insects are not damaged during the process of dehydration. (Clearing agents or staining is not necessary as the delicate and chitinised parts of these midges are sufficiently clear).

DISSECTION AND MOUNTING

Keep a drop of not very thick canada balsam on a clear slide. Transfer the midge to be dissected and mounted into this canada balsam. Note the length of the entire insect before you attempt doing anything else. Closely observe the midge in canada balsam with the help of the Bionocular microscope and make sure that all the parts of the midge are intact. Take two fine pins mounted on handles or one fine pin and an other thin blade also mounted on handles. With the help of these and the bionocular microscope, separate one of the antennae from the head of the midge. While separating the antenna, take care that the basal segments (scape and pedicel) are also detached from the head. Slowly and without applying any pressure, move this detached antenna to one side in the drop of canada balsam, which by now has well spread out. Having separated the antenna, the next part to be separated is the head. This is best done by gently pressing the thin blade on the neck region. Take this head also to one side in the canada balsam and adjust it in such a way that the palpus is extended and its segments are clearly visible for study. Then separate one of the wings from its base on the thorax in the same manner. Keep this wing also at a proper place in canada balsam, after removing the folds, if any. Then separate the three legs of one side and keep them all near each other so that the same can be recognised afterwards as the fore, mid and hind legs without much difficulty. Then separate the tip of the abdomen with the help of the piece of blade fixed in a handle. This is best done by gently pressing the blade over the abdomen, a little before the extreme tip. The ovipositor or the genitalia will get nicely separated. Move this bit also to a suitable place on the

slide and arrange it in such a way as the various parts are clearly visible. Then leave all the dissected parts in the canada balsam for some time during which it will get slightly hardened fixing the various parts in the desired positions. When you are sure that these dissected parts will not be disturbed, add the desired quantity of canada balsam and prepare the slide. Now the midge is dissected and isready for study. Number this slide and enter all the details in the register. Keep the slide for drying.

Similar process can be done on the cover slip also ; but it will not be very convenient as the coverslip is small.

Any other mounting medium like the euperal can be used. It is found through experience that canada balsam is the best for our conditions.

Any other method of dissecteing the midge may also be adopted. In such a case there is every danger of losing one or the other parts after dissection.

IDENTIFICATION

Examine the slide under any good microscope. Make sure that all the dissected parts are clearly visible and can be studied. In case the parts are not very clearly visible, apply gentle pressure on the coverslip, keeping in view that any amount of uneven pressure will damage the cover-slip and the slide as well.

Make use of the standard keys for the subfamilies, tribes and genera and identify the midge first to the sub-family and tribe. After identifying the midge upto the tribe level, dispel the doubts, if there be any. Then proceed again with the help of the standard keys for the generic identification of the midge. Now there may be some difficulty like the midge on hand partly agreeing with one genus and partly also with another genus. In such a case, run the material once again and also compare it with the original descriptions of both the genera. If you are satisfied with the identification in this manner, then decide to which of the genera the midge in question belongs. However you are to keep in view that both the sexes of the midge are to be studied simultaneously for the generic identification. Then proceed further and get at the key for the species of that particular genus and proceed in the same manner as you have done for the generic identification. Finally campare the midge in question with the original description of the species or that of the closely allied species. It is actually here the data yau have collected Viz. Host Plant, etc. will be helpful to you. If you decide that the midge under study is a new one, make notes as to its relationship with the allied species, already known. Also draw up the differences between the midge under study and the one it closely resembles.

You are cautioned here that your identifications, when you are a beginner may not be very correct and that you should take special care to declare any midge as new. To avoid any trace of incorrect identification, it is always preferable to consult a specialist.

Having finalised the identification with the help of a specialist, describe the midge in detail, if it be a new one, on the lines as others have done before. In the case of new midges, it is necessary that your description is to be supported by diagrams. Thus make camera lucida sketches or take photographs of the dissected parts of the midge. When your description and diagrams are ready, select a suitable name in accordance with the International Rules of Zoological Nomenclature. If the midge happens to be a known one, suppliment the original description with your notes.

CONCLUSIONS

(*i*) Adopt the more suitable method for collecting midges, (*ii*) Make sure that you have wet preservations in addition to prepared slides, (*iii*) Dissect on the slide more complete midges of both sexes, (*iv*) Mount the dissected parts in a way that your study of the same in easy, (*v*) Identify the midge carefully, without haste, making use of the standard keys and (*vi*) Describe the midge bringing out all the details and also supported by figures.

ACKNOWLEDGMENTS

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PEDO-ECOLOGICAL ZONES AND SOIL-TYPES OF THE SUTLEJ-TONS HIMALAYAN REGION

By

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Edaphic Ecology :

Soil is a composite living organism, which evolves in an ecological milieu. There is an unbreakable and continuous functional inter-relationship between soil and its surrounding environment. The functional inter-action between soil and milieu brings about changes not only in the composition and properties of soil, but also in the dynamics of the physical, chemical, and biological processes of soil-morphology.

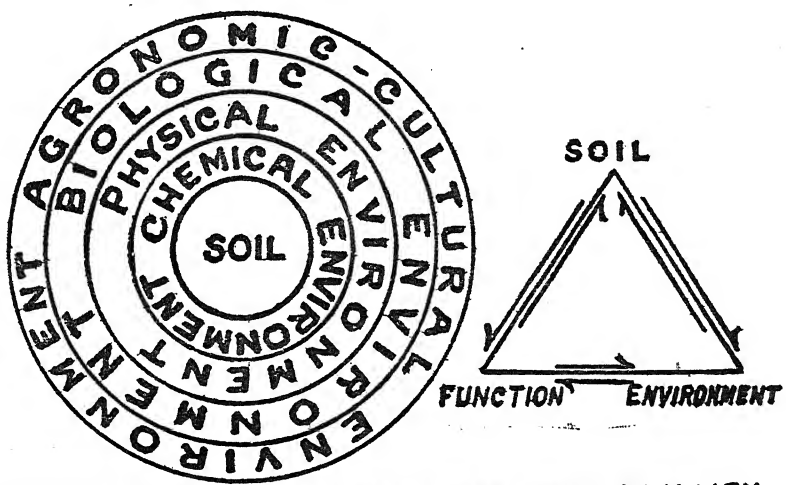


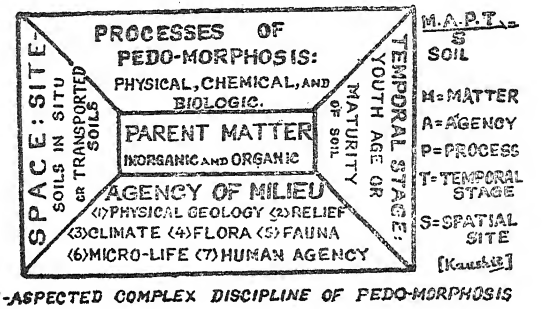
Fig. 1

In every stage of pedomorphosis, every pedo-ecological region or locality maintains a triadic balance between soil, function, and environment—both natural and cultural.

Not only the chemical composition and physical geology of the parent rocks (the joint-planes, compactness or porosity, hardness, etc.), but also the relief and

configuration of the region play a definite role in soil-formation. Climatic agencies predominate the effects of geology and chemical-built in the long run; and the biological factors comprising vegetal cover, animals, micro-life and man complete the further steps of pedomorphosis.

Thus, soil is the end-product of the physical, chemical, biological and cultural factors which act and react together in pedo-genetic processes. It develops in space, through differential temporal stages, under the action of pedo-agencies, in an ecological setting. The edaphic ecology is a five-aspected complex discipline.



FIVE-ASPECTED COMPLEX DISCIPLINE OF PEDO-MORPHOSIS

Fig. 2

The writer has studied the edaphic ecology of the Sutlej-Tons Himalayan region. Its chief features are given in the sequel.

Geographical Environment :

Location—Sutlej-Tons Himalayan region comprises the eastern part of Himachal Pradesh, lying between 30° 45′—32° 10′ N latitudes and 77° 2′—78° 55′ E longitudes.

It exists in the Inner Himalayas. On its eastern skirt lies the Shipki Pass in the Sutlej Valley, on the border between India and Tibet; and on its western skirt lies the Simla-Solon ridge. This region consists of two districts of Himachal Pradesh: the Kinnaur district lying in the east of Dhaula Dhar range, and the Mahasu district in the west of it.

Topography : its effect on soils of the region :

Sutlej-Tons Himalaya is a region of awe-inspiring grandeur with its ice-peaked ranges, prodigious rides, roaring streams and luxuriant growth of forests. Its rivers have carved deep gorges in their serpentine valleys. Innumerable streamlets have formed a network of vales and dales; and the orographic trendlines criss-cross the whole region. The Great Himalayan Range runs diagonally across the Kinnaur district in NW to SE direction.

Kinnaur is drained by the Upper Sutlej, with its affluents Spiti, Ropa, Tidong and Baspa. Mahasu has two drainage systems: Sutlej basin in its northern part

and Tons basin in its southern part. The water-parting divide between the Sutlej and Tons is a high range, joining Simla with the Dhaula Dhar range.

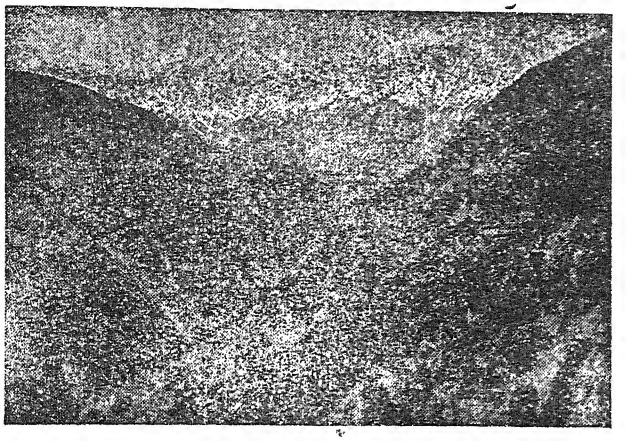


Fig. 3. The Ice-capped Range, which crosses the Kinnaur District diagonally. (Photo by Kaushic).

The average relief of the inhabited valleys of this region varies between 1,220 and 3,962 meters above sea-level. Under the force of gravity, there occur colossal landslides and avalanches. Intensive weathering, torrential rains and extensive grazing have led to a heavy soil-erosion; and the soils are usually shallow, gravelly and poor in fertility.

Because of the rejuvenation of topography, effected by the intermittent upheavals of the Himalayas, there have formed beautiful river-terraces in the valleys. It is on the river-terraces, alluvial fans, and concave slopes that the most fertile soils of this region have developed. River-terraces are the best cultivated lands.

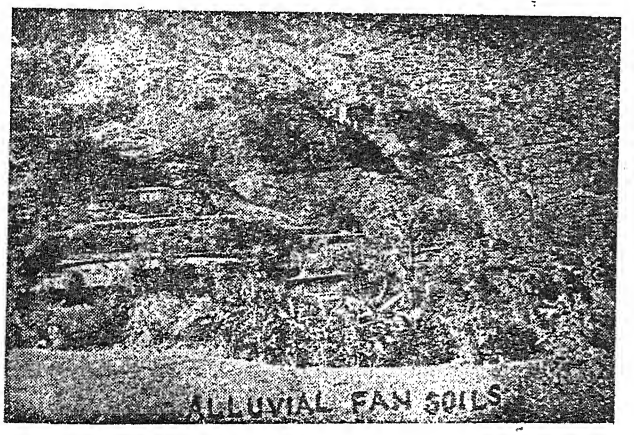


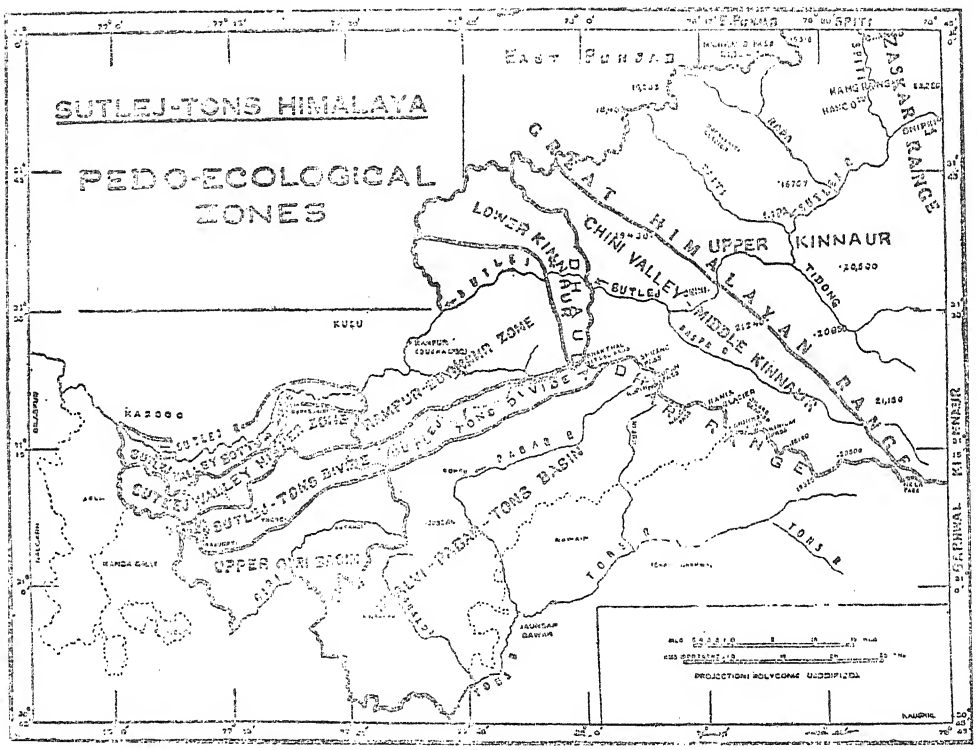
Fig. 4. Alluvial fans have well developed fertile soils. The plate shows the alluvial fan of Basantpur in the Sutlej Valley.

(Photo by Kaushic).

The effect of configuration on the accumulation or erosion of soils is of deterministic nature. Even a small accumulation of soil is potential in attracting human habitation there, howsoever small a human settlement may grow—a hamlet or even a homestead. The concave slopes are inhabited because they have deeper soils than the convex slopes, which are usually denuded and devoid of habitation.

Climate :

Climate is the active soil-former, and it tries to have an upper hand than even the parent material, to which soils show close affinity in the Himalayas because of deep erosion. The climate differs according to altitude; and micro-climate differs according to the aspect of the slope (sunny or shady), location of spurs and configuration. The altitudinal climato-vegetative zones coincide with the pedo-ecological sub-regions described in the sequel.



5. Map of the Sutlej-Tons Himalayan region, showing the Pedo Ecological sub-regions.

Pedo-Ecological Sub-regions :

According to the integrated effects of altitude, climate, and natural vegetation, there are 9 major pedo-ecological sub-regions in the area studied.

Pedo-ecological sub-region	Climato-vegetative Zone; and its soils	Altitude in meters	Mean temperature C.			Annual rainfall in cms.
			Annual	June	January	
Soni-Kumarsain Sutlej valley bottom zone	Warm subtropical; Yellow-grey forest soils	762-1,067	18-20	26-28	10-12	76-89
Soni-Kumarsian Sutlej valley higher zone	Cool subtropical; Grey-brown humid forest soils	1067-1829	14-18	21-26	7-10	102-127
Pabar-Shalvi Tons basin	Warm temperate to cool temperate; Grey-brown mixed forest soils	1219-2134	13-17	21-25	6-9	102-152
Upper Giri basin	Warm temperate to cool temperate; Grey-brown mixed forest soils	1295-2210	13-16	20-24	5-8	89-102
Sutlej Tons divide	Cool temperate to cold climate; Grey podzolised acid soils	2134-3048	8-13	15-20	1-5	127-157
Rampur-Bushahr sub-region	Subtropical Climate; Brown deciduous forest soils	914-1931	12-19	20-27	6-11	30-89
Lower Kinnaur	Cool temperate to cold climate; Grey brown mixed acid soils	1829-2438	10-13	18-21	3-7	76-89
Middle Kinnaur (Chini Valley)	Cold climate; Grey podzolised acid soils and glacial-drift soils	2438-3048	8-10	16-18	1-3	38-51
			Maximum precipitation in winter, in the form of mostly snowfall.			
Upper Ninnaur Alpine Zone	Alpine climate; Montane meadow and glacial soils	3048-4115	1-8	6-16	6 to 0	15-38
			Winter maximum, in the form of snowfall.			
	(Perpetual snow above 4575 meters—No soils.)					

Differing soils :

The Himalayan soils differ not only from one sub-region to another, but even in the same sub-region and on the same slope there are differing belts of soils from velley-bottom to the ridge-top, although erosion tends to similarise them. Soil layers are thicker on lower parts of the slopes, in valley bottoms, depressions, on flat-terraces, and scree deposits. Soil layers are also deeper on north-facing slopes, because the southern slopes are more exposed to the atmospheric agencies ; human agency too has been using the axe and spade on the southern slopes—human habitations exist on the sunny aspects (south-facing), and field-terraces lie both above and below the villages ; man has deforested these sunny alopes for making fields, obtaining fuel and fodder, and has grazed his stocks of animals over them.

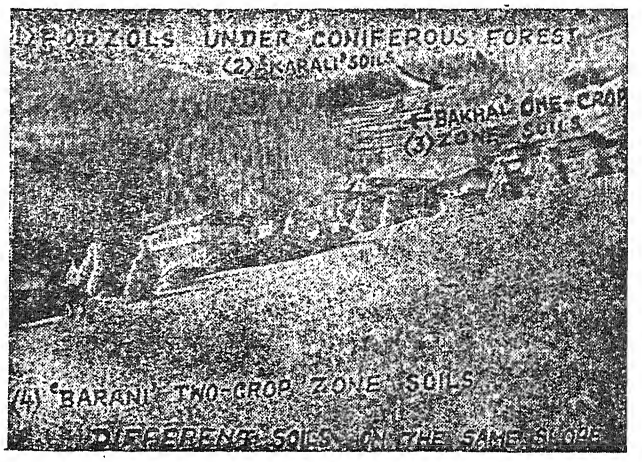


Fig. 6. Differing soils on the same slope :

(1) Podzols under coniferous forests ; (2) 'Karali' soils on unterraced 'karali' lands just below the forests ;

(3) 'Bakhal' one—crop zone soils of unirrigated terraces;

(4) 'Barani' two-crop zone soils on terraces lying below the village. (Photo by Kaushic).

The field-terraces of a village extend from the valley-bottom (sub-tropical zone) to the forests (cool temperate zone) or even ridge-top (cold zone). And, the soils of these different zones, on the same slope, represent different types. The upper-most fields have soils with pH as low as 5·5 or 5·0. They grow only one crop a year in the summer season ; and during the winter they remain buried under snow. Soils of the one-crop zone are coarser and more stony, while the valley-

bottoms grow two crops a year with their more fertile soils, which are less acid (pH 6·5 to 7·5).

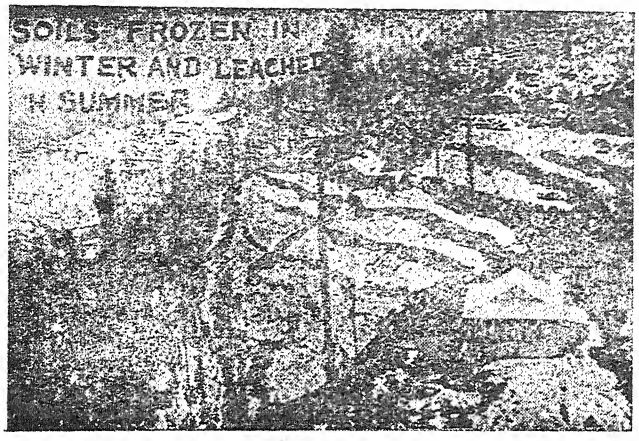
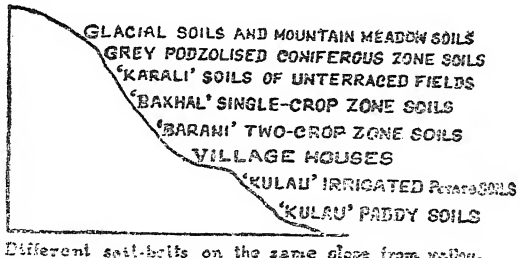


Fig. 7. Soils which freeze in winter and leach in summer: Field-terraces lying under snow-cover during winter, in the cool-temperate zone and cold zone above 2438 meters. (Photo by Kaushic).

Sample-Survey :

The writer collected samples of soils from the different sub-regions on random-sampling basis and also stratified-sampling basis. The results of some samples from each pedo-ecological sub-region are given in the sequel. The zone of maximum human settlement lies between 914 and 2134 meters. A village is usually situated on the mid-slope of a spur. The fields lie both above and below the village; and soils of the higher lying fields differ from those of lower lying ones. Take for example village Chiyog, in the Upper Giri basin. Its fields extend from 1524 to 2286 meters. There are 5 different crop-zones belonging to the same village. The upper-most part in covered with forests and pastures. Below the forest belt, there is unterraced 'Karali' land, which is cultivated intermittently after every two or three years. Below the unterraced 'Karali', there are field-terraces given to unirrigated crops. These unirrigated terraces are called 'Bakhal' lands. The upper part of the 'Bakhal' is one-crop zone, growing phaphra (*Fogopyrum tataricum*) in summer and lying fallow in winter. The lower of 'Bakhal' is called Barani'; it grows two crops a years—mountain millete as marsa (*Amaranthus frumentaceus*), koto



7 Different soil-belts on the same slope from valley-bottom to ridge-top.

Fig. 8

Fagopyrum esculentum), kauni (*Pauicum italicum*), cheena *Panicum miliacum*), or koda *Elucine coracana*) in summer, and wheat or barley in winter. Below the 'Barani' terraces, there lies the village. Then, there are irrigated field-terraces; called 'Kuiau' lands, which grow maize, gahath *Dolichus biflorus*), beans, and potato in summer, and wheat or mustard in winter. Near the bottom of the valley, there are plots for paddy in summer and wheat in winter.

Difficulty in Regional Classification :

It is very difficult to prepare a regional classification of the Himalayan soils, because the ecological factors and pedo-processes differ from locality in locality, and also because the soils are mostly in their immature stage (because of the heavy erosion and transportation of the finer contents to the valley-bottoms and further down to the foot-hill zone). The influence of the parent material is predominent. Under forests, the profiles are usually more mature than on the field-terraces.

Fig. 9. Lithozols, Podzols, and grey-brown forest-soils-under-cultivation, at Chiyog in the Giri basin. (Photo by Kaushic).

The above plate shows the influence of parent material and climato-vegetative effect on the soil-formation of Chiyog valley in higher tract of the Giri basin. Three different soils—lithozols on the barren slopes, podzols under the coniferous forests, and grey-brown cultivated soils on the fluvio-glacial deposits—are distinctly demarcated there. Soils of the valley bottom are still different. Such local and micro-local differences make a very precepitous path to be followed by the soil-scientist in demarcating the pedo-ecological zones, or sub-regions.

Therefore, it is necessary that before the final mark of sub-divisions, several thousands of samples be collected and analysed. However, the sample collected and analysed by the author give a bird's eye-view of the general classification of the soil-types and their different pedo-ecological sub-regions.

Results of analyses :

Below are given the results of the mechanical and chemical analyses of some of the samples from the different sub-regions.

Pedo-ecological sub-region	Sample No.	Soil colour	Texture	pH	CaO	N	P_2O_5	K_2O
					%		%	%
Soni-Kumarsain Sutlej-valley-bottom zone	1	Dark brown	Loam ...	6·50	1·78	0·20	0·08	0·55
	2	Grey brown	Clayey loam	7·50	3·85	0·25	0·30	6·60
	9	Grey brown	Silty loam ...	6·70	1·26	0·24	0·14	0·48
	10	Yellowish brown	Loam ...	6·35	1·98	0·36	0 25	0·28
Soni-Kumarsain Sutlej-valley upper zone	16	Pale brown	Light loam...	6·30	0·84	0·23	0·32	1·05
	17	Brownish grey	Loam ...	6·65	0·75	0·52	0·80	1·50
	19	Brown ...	Clayey loam	6·35	0·64	0·32	0·50	1·10
	23	Yellowish brown	Sandy loam	6·30	0·60	0·28	0·30	0·80
Pabar-Shalwi zone of the Tons basin ...	28	Brown ...	Silt loam ...	6·11	1·40	0·20	0·64	1·90
	30	Brown ...	Clayey loam	6·70	0·43	0·15	0·22	0·45
	31	Brown ...	Loam ...	1·45	0·64	0·32	0·50	1·10
	34	Grey brown	Silt loam ...	7·3	2·14	0·60	0·75	0·90
Upper Giri velley	41	Grey brown	Silt loam ...	6·4	0·38	0·23	0·28	0·55
	42	Brown ...	Sandy loam	6·2	0·25	0·19	0·32	0·20
	45	Light grey ...	Loam (Podz)	6·3	0·28	0·30	0·18	0·48
	47	Brown ...	Loam ...	6·8	0·42	0·23	0·25	0·80
*Sultej-Tons divide	54	Pale brown...	Clayey loam	6·2	0·54	0 18	0·15	1·16
	55	Grey brown	Loam ...	6·6	0·38	0·32	0·28	0·93
	57	Pale brown...	Silt loam ...	6·3	0·40	0·42	0·20	0·75
	58	Grey ...	Loam ...	6·1	0·68	0·33	0·80	0·40
Rampur-Bushahr zone	62	Dark brown	Sandy loam	6·2	0·23	0·38	0·15	0·30
	63	Grey ...	Clayey loam	7·5	1·80	0·60	0·18	0·50
	66	Brown ...	Loam ...	6·4	0·68	0·52	0·48	1.00
	69	Grey ...	Fine loam ...	6·7	2·20	0·34	0·26	0·78
Middle Kinnaur (Chini Valley)	76	Grey brown	Loam ...	6·4	0·43	0·35	0·15	2·12
	77	Brown ...	Sand loam ...	6·2	0·48	0·29	0·18	1·85
	79	Light brown	Loam ...	6·4	0·38	0·25	0·29	2·08
	80	Yellow brown	Loam ...	6 5	0·52	0·18	0·35	1·98
Upper Kinnaur Alpine zone ...	86	Pale brown...	Sandy loam	6·1	0·27	0·18	0 21	0·92
	39	Pale brown...	Sandy loam	6·0	0·40	0·22	0·29	0·78

Analysis indicates that the soils show a considerable affinity to the parent material. In the Great Himalayan Zone, soils on the upper parts of the ridges have developed from granitic rocks. They contain fair quantities of potash, but their CaO % is very low. The zone-between 3048 and 4115 meters remains buried under snow for more than 7 months in a year. Its soils are 'glacial soils', frozen

*An analysis of the soils of a part of this sub-region, known to the author, is that given by B. K. Sharma, K. S. K. Rao, R. N. Paul and Pushkarnath—Nutrient Status of soils of N. E. S. Block, Theog, Journal Ind. Soc. Soil Sc. Vol. 4, No. 4 (1956) pp. 241-246.

and gritty. Landslides are a common occurence in the winter. The 'A' horizon of these soils centains ash-grey leached layer, mostly of hydrous mica, illite, etc. In this Alpine zone, there are summer pastures of trans-humance. The herds of sheep, goats and cattle are sent to graze over these pastures in summer from June to September. These pastures provide very nutrient and succulent fodder, although these 'Montane meadow soils'[1] contain large quantities of gravel and grit. Soils of many localities in Upper Baspa Valley, Hango-Chango, Ribba Dhar, and Spiti Valley are cold clays supporting good pastures.

Between 2134 and 3200 meters, there are podzolised acid soils under coniferous forests. In Mahasu district, in the west of the Dhaula Dhar Range, on the Sutlej-Tons divide range, the soils have developed under the influence of summer monsoonal rains and winter cyclonic rains and snowfall. In the east of Dhaula Dhar, in Kinnaur district, the podzolised soils of the coniferous zone have developed in the monsoon-free region. The 'A' horizon of these temperate zone soils are slightly leached and greyish brown in colour. In many localities it is light grey, because of an abundance of silica, after the leaching of iron and aluminium from 'A' to 'B' horizon. By eluviation, it loses most of its clay and colloidal contents. A_2 horizon contains hydrous mica, illite, and kaolinite. 'B' horizon is illuviated from above; it is dark brown or yellowish brown in colour. Iron has tended to become oxidised. The 'C' borizon in composed of glacial-drift, or uneroded mica-schists or granitic intrusive rocks. There are mixed soils of glacial and fluvio-glacial origin; and many soils have developed out of talus or surface-creep from higher slopes.

Between 1524 and 2286 meters there are greyish podzolic soils under conifers and grey-brown or brown soils under deciduous forests. The soils of this zone are suitable for the cultivation of koda (*Eluaine coracana*), tulsi (*Amaranthus frumentaceus*), maize, wheat, barley and potato. Between 914 and 1524 meters, there are brown, yellowish brown and greyish brown humid subtropical soils, whieh are suitable for the cultivation of maize, potato, mandua, wheat and hilly rice. Below is given a very general sketch of the different altitudinal-zone soils suited to different crops and vegetation :—

Altitude in meters	Crops grown	Vegetal cover and soils
Above 4420 ...	No crop ...	Ice and snow.
4115-4420 ...	No crop ...	Frozen glacial soils.
3200-4115 ...	Phaphra, naked barley and potato	Alpins pastures; Montane meadow soils.
2134-3200 ...	Amaranthus, wheat, barley, potato, and fruits—apples, apricots, pears, etc.	Coniferous forests; podzolised acid soils.
1524-2134 ...	Koda, cheena, maize, beans; potato, wheat, barley and mustard.	Deciduous and mixed coniferous forests; Grey brown forest soils.
914-1524 ...	Rice, maize, wheat, hilly millets.	Humid subtropical forests; Yellow-brown and yellowish grey soils.

1. The same nomenclature for the Alpine Zone pasture-soils is used by Schokalskaya, Z. J., in her famous work—"The Natural Conditions of Soil Formation in India ; Contributions to the Knowledge of Soils of Asia, No. 2 (Leningrad)—1932, pp. 140-155."

Potato soils and fruit-soils:

Special mention is needed by potato, which grows well on these soils of all the sub-regions upto 3962 meters. Potato is an acid-tolerent crop, and podzols of fine texture, when manured with litter and dung, yield heavy crops of potato. Therefore, the farmers have been paying more and more attention to the cultivation of potato; and it has become a very important cash crop of Mahasu district, which exports (via Simla) seed-potato to other states of India. Area under potato cultivation in the Sutlej-Tons Himalayan region is about 55,000 acres and yeild about 80 mds. to 100 mds. per acre.

The soils of this region are, though poor for raising food-grain crops, yet very suitable for horticulture. Apples, peaches and apricots would grow in the whole region between 1524 and 3048 meters.

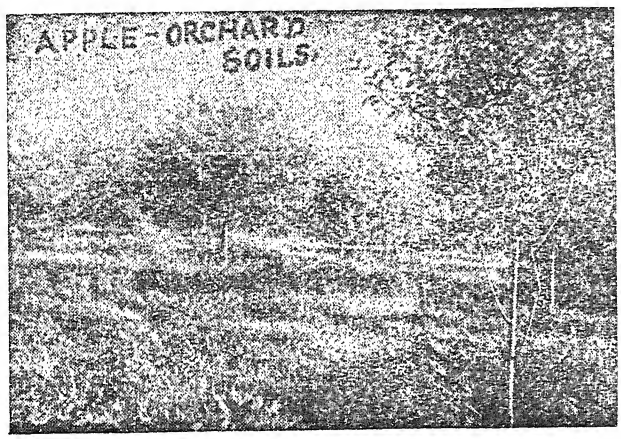


Fig. 10. Apple soils of Kothgarh-Thanadhar area. Kotgarh apples fetch high prices in Calcutta market (Photo by Kaushic).

Kinnaur district is a mansoon-free area of Mediterranean type climate, with winter precipitation and summer sunshine. Its soils join hands with the climate in their suitability for the horticulture of grapes, raisin grapes, almonds, apricots, peaches and apples. Vine is an indigenous plant of Kinnaur. Dry fruits which flourish well are walnut, hazelnut, neoza (chilgoza), and pista.

If cultivated on scientific lines, as the Government of Himachel Pradesh is proposing to do, this area of the Sutlej-Tons Himalaya will become the most important producer of potato and fruits in the whole of India. If transport routes are established with Kinnaur district, then, Kinnaur grapes, raisins, currants, almonds, apples, apricots, dry fruits, and even its famous red wine 'Shibu' would be available to the plains of India. Then, India will save her millions of rupees which are now being spent on the import of these commodities.

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CONTENTS

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